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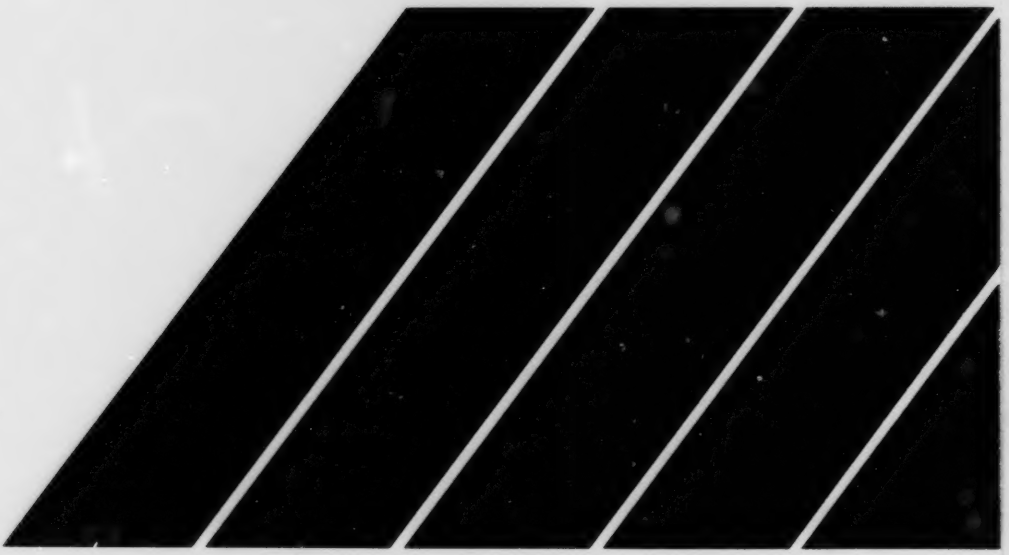
ECONOMIC AND PROCESS OPTIMIZATION OF ETHANOL PRODUCTION BY EXTRACTIVE FERMENTATION



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ECONOMIC AND PROCESS OPTIMIZATION OF ETHANOL PRODUCTION BY EXTRACTIVE FERMENTATION

PREPARED FOR:

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PREFACE

This report is intended to demonstrate the economic advantages of ethanol production by Extractive Fermentation over the best alternative technology currently available. A parallel effort involving experimental work as well as process simulation and optimization has been employed. The significance of this work lies in the potential commercialization of this Canadian technology by private industry. This report reveals that considerable savings can be realized by using Extractive Fermentation technology for ethanol production, for both "grass-roots" and retrofit scenarios. With recent developments in the Middle East, as well as increasing environmental concerns, interest in renewable, domestic fuel alternatives and oxygenates such as ethanol will certainly increase, and it is anticipated that Extractive Fermentation will be a key technology in the expansion of the fermentation ethanol industry.

ABSTRACT

The economic advantages of Extractive Fermentation on an industrial scale compared to the best alternative technology currently available was demonstrated by computer simulation. The simulations were based on a plant capacity of 100 x 10^6 L/y of azeotropic ethanol. When compared on a grass-roots basis, the economically optimum ethanol selling price for a conventional plant, utilizing a continuous-stirred tank fermentor with cell recycle, was 45 ¢/L ($CAN, 1989) for a 15% return on investment. A grass-roots Extractive Fermentation plant produced a selling price of 39 ¢/L with the same 15% return on investment. Thus, a grass-roots Extractive Fermentation facility would produce ethanol at a savings of approximately $6 million/y. Even greater cost savings (as much as 15 ¢/L) could be realized by retrofitting an existing plant with Extractive Fermentation technology, while at the same time more than doubling plant capacity without extensive modifications.

The simulation results were verified with a fully integrated, computer controlled Extractive Fermentation Process Demonstration Unit based around a 7 L fermentor. When operated with a synthetic glucose medium and using *Saccharomyces cerevisiae*, the optimum operating conditions were in agreement with those predicted by computer simulation. The system was also operated with natural substrates (blackstrap molasses and grain hydrolyzate). As well, preliminary tests with the organism *Zymomonas mobilis* were carried out under Extractive Fermentation conditions.

EXECUTIVE SUMMARY

Extractive Fermentation is a process in which a biological product is extracted directly from a fermentation vessel as it is produced, thus removing any potential cell-inhibiting effects of that product. The kinetic advantages of ethanol production by Extractive Fermentation over conventional fermentation have been well documented (Daugulis, et al., 1987; Kollerup and Daugulis, 1987); however, the economic advantages of the process were previously unknown. Thus, a thorough investigation into the economics of Extractive Fermentation was undertaken, with the ultimate goal of transferring the technology to private industry.

The economic analysis was conducted by computer simulation using PROCESS™ (Simulation Sciences Inc., Fullerton, California) which is a commercial simulation package primarily used in the chemical, petroleum, and synthetic fuels industries. The conventional plant simulation was modeled on the best available fermentation technology currently in use, which is a continuous-stirred tank fermentor with cell recycle. The Extractive Fermentation system was also modeled on this type of fermentor, but with the added Extractive Fermentation technology. Both plants were based on a production capacity of 100×10^6 L/y of azeotropic ethanol, and common design methods and costing techniques were used. The economics involved in retrofitting an existing ethanol facility with Extractive Fermentation technology were also investigated. The accuracy of the simulation results with respect to fermentor conditions at the predicted optimum flowrates were tested on an Extractive Fermentation Process Demonstration Unit using a 7 L fermentor.

Based on a fermentor cell concentration of 75 g/L (dry wt), the economically optimum conditions predicted by PROCESS™ for the conventional plant were an aqueous dilution rate of 0.20 h^{-1} and a feed concentration of 18% (wt) fermentables. Under these conditions an ethanol selling price of 45.06 ¢/L ($CAN, 1989) would be required for a 15% return on investment. For the Extractive Fermentation simulations the feedstock was fixed at 41.5% (wt) fermentables, because this can be fermented to near complete conversion with Extractive Fermentation and because this concentration of fermentables is normally present with some natural feedstocks, such as molasses. The simulations produced a lowest cost "trough"; that is, there were several economically optimum points, each corresponding to a set of aqueous and

solvent dilution rates. For example, at aqueous and solvent dilution rates of 0.15 h^{-1} and 1.25 h^{-1}, an ethanol selling price of 38.69 ¢/L would yield a 15% return on investment. That corresponds to a savings of approximately $6 million/y over the conventional plant. Moreover, the results indicated that an Extractive Fermentation plant could be built for approximately the same capital cost as a conventional plant.

The Extractive Fermentation plant design was configured to be as similar to the conventional plant as possible in order to facilitate the retrofitting of a conventional plant with Extractive Fermentation technology. Such a retrofit scenario was simulated by PROCESS™. The results indicated that an ethanol selling price of 29.44 ¢/L would be required to maintain the same level of profit as that achieved with the conventional plant plus an additional 15% return on the retrofit equipment. Based on the extra revenue generated by the cost savings, the payback period for the retrofit equipment would be approximately 1.8 months. The simulations also predicted that by retrofitting an existing plant with Extractive Fermentation technology, it is possible to more than double that plant's capacity.

The operating conditions predicted by the computer simulations were tested on an Extractive Fermentation Process Demonstration Unit (PDU) utilizing a 7 L fermentor and upgraded equipment, and using a strain of *Saccharomyces cerevisiae* as the fermenting organism. The results showed optimal flow conditions to be 0.12 h^{-1} and 1.25 h^{-1} for aqueous and solvent dilution rates, respectively; these were in reasonable agreement with the predicted values of 0.15 h^{-1} (aqueous dilution rate) and 1.25 h^{-1} (solvent dilution rate).

Two runs were performed to investigate how the PDU would perform with natural substrates, namely blackstrap molasses and grain hydrolyzate. Approximately 96% conversion of the hydrolyzate (335 g/L fermentables) was achieved at aqueous and solvent dilution rates of 0.15 h^{-1} and 1.25 h^{-1}, respectively. The molasses proved more difficult to ferment, as only 82% conversion was achieved at a substrate concentration of 287 g/L fermentable sugars and an aqueous dilution rate of 0.10 h^{-1}.

A run with *Zymomonas mobilis* as the fermenting organism produced encouraging results. At a cell concentration of only 3.2 g/L and a dilution rate of 0.08 h^{-1}, over 85% conversion of a 459 g/L feedstock was achieved.

Cell recycle could not be effectively employed since it was discovered that stillage recycle to the fermentor greatly reduced cellular activity.

The economic work and experimental verification provided the groundwork for technology transfer to the private sector. Efforts at licensing the technology continue to be made. Negotiations with Monsanto Enviro-Chem (a wholly-owned subsidiary of Monsanto Corporation, St. Louis, MO) and JonTon Alcohol (Edinburg, Texas) are ongoing. A plant design to retrofit the JonTon plant with Extractive Fermentation technology is being developed. Several other firms, both in North America and abroad, have also shown interest in acquiring Extractive Fermentation technology.

In light of the recent uncertainty in foreign energy supplies, and the increasing public awareness of renewable resources and environmental protection, it is not surprising that private firms have expressed their interest in this technology. More work, however, is required in the areas of natural substrates, namely, molasses and grain hydrolyzate, as these would be the feedstocks used in industry. As well, the potential of *Z. mobilis* in an Extractive Fermentation system has not yet been fully explored. It is believed that a pilot-scale system based on a 150 - 300 L fermentor would provide substantially improved results and would allow integration of Extractive Fermentation with molecular sieve technology. The latter technology has the potential for producing anhydrous ethanol without distillation. A larger system would also act as a demonstration unit to potential licensees, as well as providing information on scale-related problems, such as formation of emulsions, effect of aeration, requirements for solvent recycle, etc. Such information would be invaluable for future industrial scale operation.

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RÉSUMÉ

La fermentation extractive est un procédé qui consiste à extraire directement un produit biologique à partir d'un dispositif de fermentation, au fur et à mesure qu'il est produit, ce qui élimine tout risque d'inhibition des cellules par ce produit. Les avantages cinétiques de la production d'éthanol par fermentation extractive, par rapport à la fermentation traditionnelle, sont bien documentés (Daugulis et coll., 1987; Kollerup et Daugulis, 1987); toutefois, on ne savait rien auparavant des avantages économiques de ce procédé. On a donc entrepris une étude approfondie des aspects économiques de la fermentation extractive, dans le but de transférer cette technologie à l'industrie privée.

L'analyse économique a été effectuée par simulation informatisée, avec PROCESS^{mc} (Simulation Sciences Inc., Fullerton, Californie), une trousse de simulation commerciale principalement utilisée dans les industries des produits chimiques, pétroliers et des combustibles synthétiques. La simulation de l'usine classique a été modélisée d'après la meilleure technologie de fermentation actuellement utilisée, à savoir un réservoir de fermentation à agitation continue, avec recyclage des cellules. Le système de fermentation extractive était également basé sur ce type de fermenteur mais auquel on avait intégré la technologie de la fermentation extractive. Les deux usines étaient basées sur une capacité de production de 100 x 10^6 L/an d'éthanol azéotrope et les méthodes courantes de conception et de calcul des coûts ont été appliquées. On a également étudié les aspects économiques de l'adaptation d'une usine existante de fabrication de l'éthanol à la technologie de la fermentation extractive. On a vérifié l'exactitude des résultats de la simulation, pour ce qui est des conditions de fonctionnement du fermenteur aux débits optimaux prévus, sur une unité de démonstration du procédé de fermentation extractive munie d'un fermenteur de 7 litres.

Si l'on se base sur une concentration des cellules de 75 g/L (masse sèche) dans le fermenteur, les conditions économiquement optimales prévues par PROCESS^{mc} pour l'usine classique étaient les suivantes : un taux de dilution aqueuse de 0,20 h^{-1} et une concentration d'alimentation de 18 % (masse) de composés fermentescibles. Dans ces conditions, il faudrait vendre l'éthanol 45,06 ¢/L ($ CAN, 1989) pour obtenir un rendement du capital investi de 15 %. Dans le cas des simulations de la fermentation extractive, on a fixé la concentration des composés fermentescibles à 41,5 % (masse) dans la charge d'alimentation parce qu'une telle proportion peut être fermentée jusqu'à conversion presque complète par fermentation extractive et parce que cette concentration de composés fermentescibles est normalement présente dans certaines charges d'alimentation naturelles comme la mélasse. Les simulations ont donné un "creux" du coût le moins élevé, c'est-à-dire que l'on a obtenu plusieurs points économiquement optimaux, correspondant chacun à une série de taux de dilution avec de l'eau et

avec du solvant. Par exemple, pour des taux de dilution avec eau et avec solvant de 0,15 h^{-1} et de 1,25 h^{-1}, on obtiendrait un rendement du capital investi de 15 % en vendant l'éthanol 38,69 ¢/L. Cela correspond à une économie d'environ 6 millions de dollars par an par rapport à l'usine traditionnelle. De plus, les résultats ont montré qu'il serait possible de construire une usine de fermentation extractive pour à peu près le même coût en capital qu'une usine traditionnelle.

L'usine de fermentation extractive a été conçue de manière à ressembler le plus possible à l'usine classique afin de faciliter l'adaptation d'une usine classique à la technologie de la fermentation extractive. Un tel scénario d'adaptation a été simulé avec PROCESS^MC. Les résultats ont indiqué qu'il faudrait vendre l'éthanol au prix de 29,44 ¢/L pour maintenir les profits au même niveau qu'avec l'usine classique plus un rendement supplémentaire de 15 % sur le capital investi dans l'équipement nécessaire à l'adaptation. En se basant sur les revenus supplémentaires générés par l'économie de coûts, la période de recouvrement de l'équipement d'adaptation serait d'environ 1,8 mois. Les simulations ont également permis de prévoir qu'en adaptant une usine existante à la technologie de la fermentation extractive, il serait possible de plus que doubler la capacité de l'usine.

Les conditions d'exploitation prévues par les simulations informatisées ont été testées sur une unité de démonstration du procédé de fermentation extractive (UDP) comportant un fermenteur de 7 L et de l'équipement adapté. Une souche de *Saccharomyces cerevisiae* a été utilisée comme organisme de fermentation. Les résultats ont indiqué que les conditions de débit optimal seraient respectivement de 0,12 h^{-1} et 1,25 h^{-1} pour les taux de dilution avec eau et avec solvant; ces valeurs concordaient raisonnablement bien avec les valeurs prévues qui étaient de 0,15 h^{-1} (taux de dilution aqueuse) et de 1,25 h^{-1} (taux de dilution avec du solvant).

On a effectué deux passages pour étudier comment l'UDP fonctionnerait avec des substrats naturels, soit avec de la mélasse et de l'hydrolysat de céréale. On a ainsi obtenu un taux de conversion d'environ 96 % de l'hydrolysat (335 g de composés fermentescibles/L) avec des taux de dilution avec eau et avec solvant de 0,15 h^{-1} et 1,25 h^{-1}, respectivement. La mélasse s'est révélée plus difficile à fermenter : le taux de conversion était de seulement 82 % pour une concentration de substrat de sucres fermentescibles de 287 g/L et un taux de dilution avec de l'eau de 0,10 h^{-1}.

Un passage avec lequel on a utilisé *Zymomonas mobilis* comme organisme de fermentation a donné des résultats encourageants. La concentration des cellules étant de seulement 3,2 g/L et le taux de dilution, de 0,08 h^{-1}, on a obtenu un taux de conversion de plus de 85 % avec une charge d'alimentation de 459 g/L. On a découvert que le recyclage des cellules dans le fermenteur ne pouvait être employé efficacement car il réduisait de beaucoup l'activité cellulaire.

Les travaux sur les aspects économiques et la vérification expérimentale ont permis de préparer le terrain en vue du transfert de la technologie vers le secteur privé. Les efforts se poursuivent en vue de la concession de licence. Les négociations se poursuivent également entre Monsanto Enviro-Chem (propriété exclusive de Monsanto Corporation, St Louis, MO) et JonTon Alcohol (Edinbug, Texas). On cherche actuellement à adapter l'usine de JonTon à la technologie de la fermentation extractive. Plusieurs autres sociétés, en Amérique du Nord et ailleurs, se sont aussi montrées intéressées à acquérir la technologie de la fermentation extractive.

Étant donné les incertitudes que suscite depuis peu l'approvisionnement en énergie à l'étranger et vu la prise de conscience du public à l'égard des ressources renouvelables et de la protection de l'environnement, il n'est pas surprenant que des sociétés privées manifestent leur intérêt à l'égard de cette technologie. Il faudra toutefois effectuer d'autres travaux dans les domaines des substrats naturels, soit la mélasse et l'hydrolysat de céréales qui seraient les charges d'alimentation utilisées dans l'industrie. De plus, le potentiel de *Z. mobilis* dans un système de fermentation extractive n'a pas encore été exploré à fond. On croit qu'un système pilote muni d'un fermenteur de 150 - 300 L donnerait des résultats nettement améliorés et permettrait d'intégrer la fermentation extractive à la technologie des tamis moléculaires. Cette dernière technique pourrait permettre de produire de l'éthanol anhydre sans distillation. Un système plus gros pourrait aussi servir d'unité de démonstration pour les preneurs de licence potentiels et fournir de l'information sur des problèmes liés à l'échelle comme la formation d'émulsions, l'effet de l'aération, les exigences relatives au recyclage du solvant, etc. De telles informations seraient précieuses pour l'exploitation future à l'échelle industrielle.

1.0 INTRODUCTION

Fermentation ethanol is an important industrial chemical, both in the pharmaceutical/cosmetic industry and as a fuel source, either as a gasoline replacement or as an oxygenate additive. Indeed it is the largest single fermentation product by volume in the world (Maiorella, 1985). It is particularly attractive in a fuel context because it is a renewable resource that can be produced domestically. In some countries, like Brazil, it has already become a major fuel source, as shown by the three million automobiles there which run exclusively on ethanol (Amorim, 1989).

Traditionally, fermentation ethanol has been produced by using microorganisms to convert simple sugars to ethanol in a batch process. Such a process is limited, however, by the problem of end-product inhibition. That is, as the ethanol accumulates in the fermentation broth, it inhibits cell activity resulting in reduced cell and ethanol production (Bazua and Wilke, 1977). As well, end-product inhibition allows only dilute feedstocks to be used, resulting in a large amount of downstream water processing, which is both energy intensive and costly, and can cause serious environmental problems.

Extractive Fermentation is a process which eliminates the problems of end-product inhibition. By removing the ethanol directly from the reaction vessel as it is formed via liquid/liquid extraction, the ethanol production rate remains high. Earlier work (Maiorella, et al., 1984a) had suggested that an industrial scale Extractive Fermentation grass-roots facility could produce ethanol at a savings of over $5 million ($ US) a year compared to the best available conventional technology for a plant capacity of 100×10^6 L/y of azeotropic ethanol. However, at that time, no working Extractive Fermentation system had yet been sufficiently developed to permit a detailed economic analysis. As well, the economic results of retrofitting an existing plant with Extractive Fermentation technology could not be explored.

A working Extractive Fermentation prototype system was subsequently developed (Kollerup and Daugulis, 1986) which demonstrated the kinetic advantages of Extractive Fermentation over conventional fermentation. Later work on a larger system with an integrated ethanol thermal recovery system verified that long-term stable operation of the Extractive Fermentation process with concentrated feedstocks is possible (Daugulis, et al., 1987). Although this work provided valuable insights into the kinetic advantages of

Extractive Fermentation, it did not detail the economic advantages of the process compared to the best available conventional fermentation technology. The optimum operating conditions of an industrial scale Extractive Fermentation facility were also unknown. Although the technology had been widely published and even patented (U.S. Patent # 4,865,973), it was unlikely that any commercialization activities would begin before a full economic analysis was undertaken.

2.0 OBJECTIVES

The principle objective of the work was to demonstrate, through computer simulation, the economic advantages of Extractive Fermentation over the best available conventional technology. This would be achieved by comparing the production cost of ethanol from an optimized grass-roots Extractive Fermentation plant to that of an optimized conventional plant. The possibilities arising by retrofitting an existing plant with Extractive Fermentation technology were also to be investigated.

A second but no less important objective was to verify the simulation results on a laboratory scale Extractive Fermentation Process Demonstration Unit which had been refitted with upgraded equipment. Operation of this system was also tested with natural substrates (blackstrap molasses and grain hydrolyzate), as these would be the feedstocks of choice on an industrial scale. The use of *Zymomonas mobilis* as a fermentation organism was also investigated.

Computer simulation and verification were designed to facilitate a third objective, namely, commercialization of Extractive Fermentation technology.

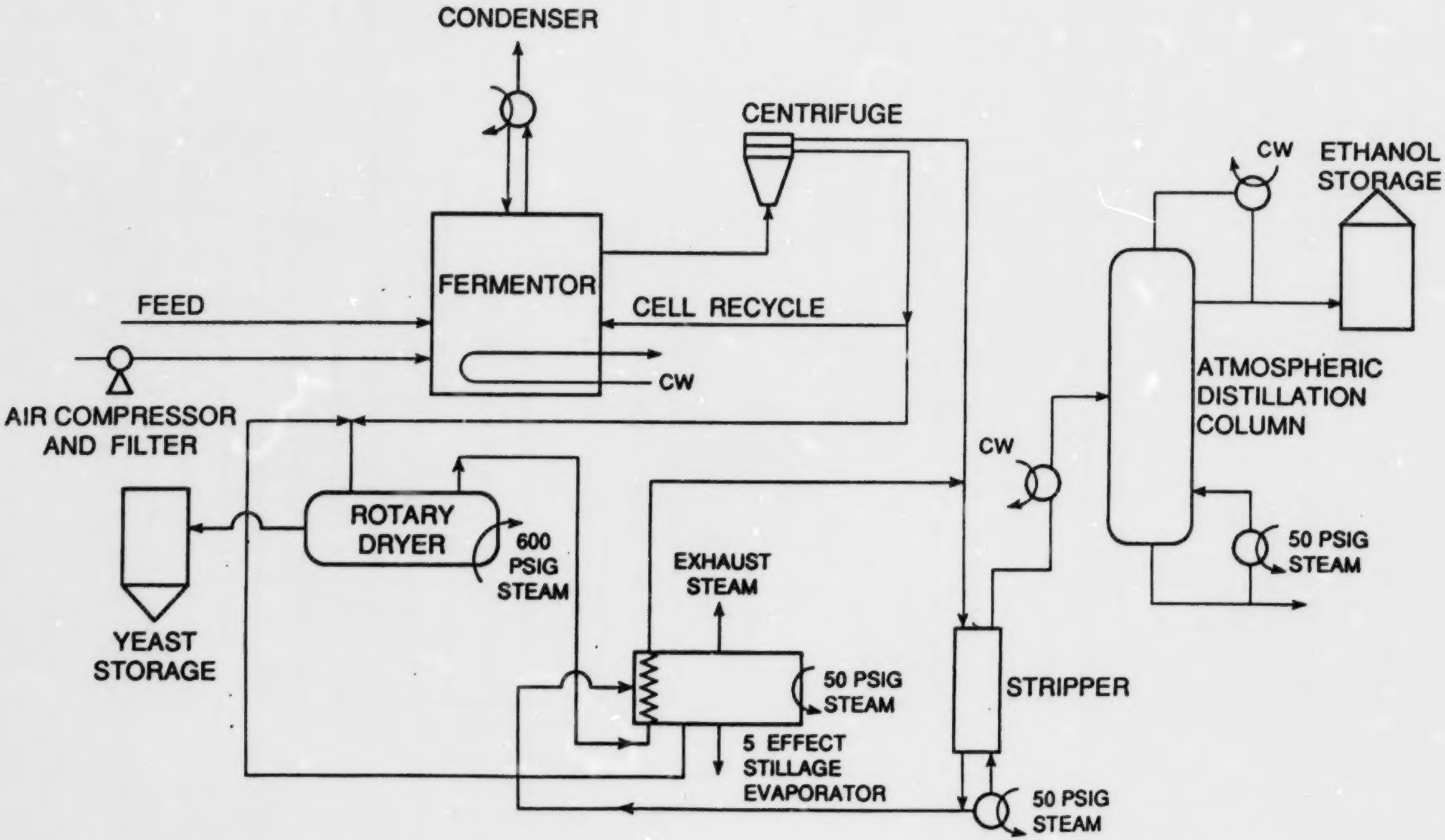
3.0 ECONOMIC SIMULATION WORK

3.1 PROCESS FLOW DIAGRAMS

The economic analysis of the Extractive Fermentation process was based on a comparison between a conventional ethanol plant design and one using Extractive Fermentation technology, with both operating at their respective economically optimum conditions. The conventional ethanol plant design, presented in Figure 3-1, uses a continuous stirred-tank reactor (CSTR) with a cell recycle system to allow continuous operation and to maintain a high cell concentration in the fermentor. Consistent with current practice, some

FIGURE 3-1

CONVENTIONAL ETHANOL PRODUCTION


CONDENSER
CENTRIFUGE
CW
ETHANOL STORAGE
FERMENTOR
FEED
CELL RECYCLE
CW
AIR COMPRESSOR AND FILTER
ATMOSPHERIC DISTILLATION COLUMN
CW
ROTARY DRYER
600 PSIG STEAM
YEAST STORAGE
EXHAUST STEAM
50 PSIG STEAM
50 PSIG STEAM
STRIPPER
5 EFFECT STILLAGE EVAPORATOR
50 PSIG STEAM

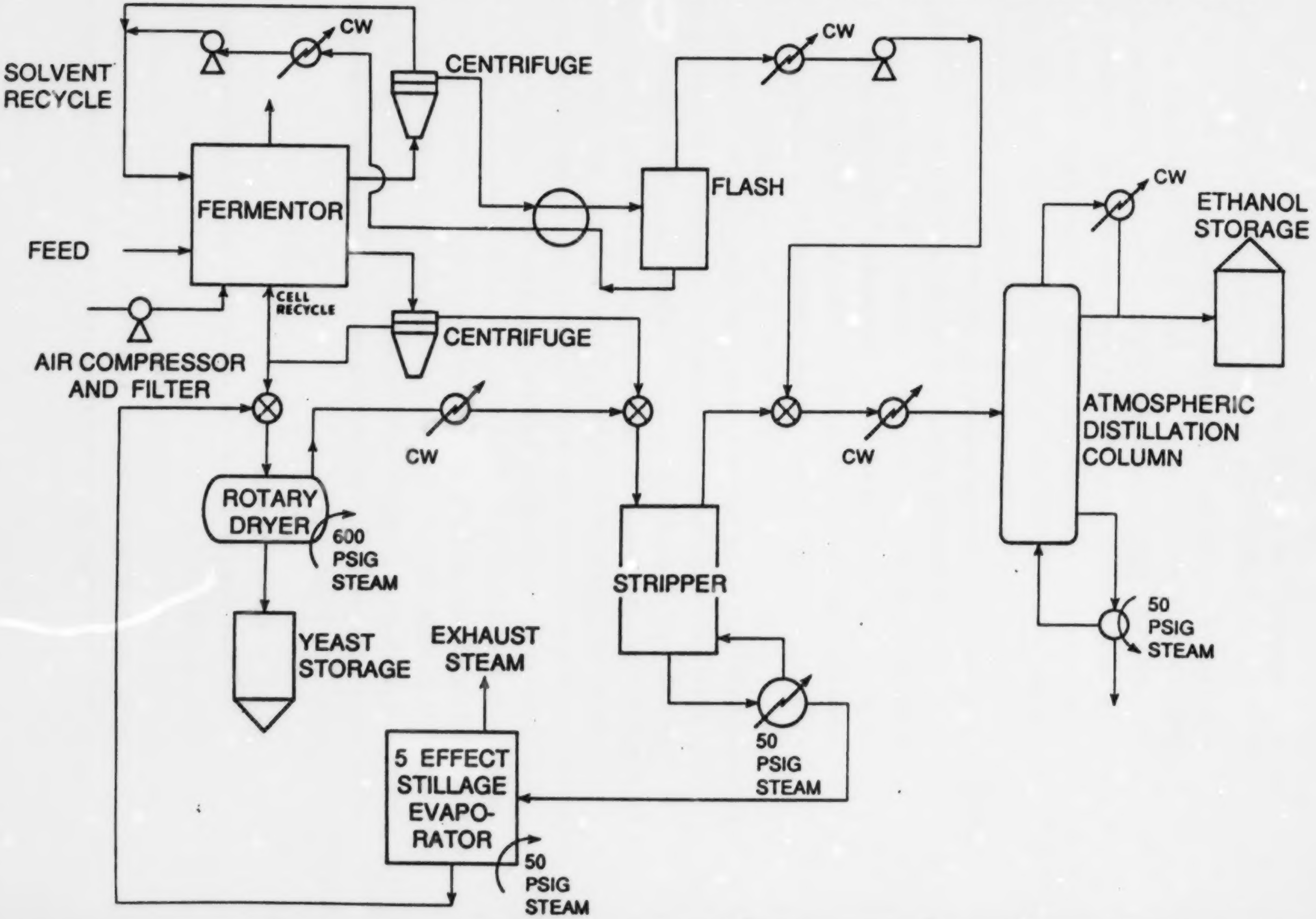
aeration is provided to maintain cell viability and functionality under high cell concentration conditions. This design was chosen because it uses advanced existing technology and provides one of the lowest ethanol production costs of all fermentation alternatives that do not employ selective ethanol removal (Maiorella, et al., 1984a). With this design, fermentables can be fed to a CSTR at a variety of concentrations (typically 18 - 20 % w/v), the optimum being determined by economic simulation. The added complication of feed sterilization is ignored because it represents a minor component in the conventional plant cost, and is likely to pose minimal problems due to the high yeast concentrations employed. The fermentation broth is centrifuged to remove the cells, with the supernatant being sent to the stripper/distillation section for ethanol recovery. A portion of the concentrated cells is returned to the fermentor for cell recycle while the remainder is sent to a rotary dryer. Once dried, the solids can be sold as a feed supplement, and thus represent a credit. The stripper bottoms, which contain various solids such as proteins and unmetabolized feed components, are fed to a five-effect stillage evaporator, where the solids are concentrated. From here, they too are sent to the rotary dryer to be incorporated with the dried yeast product as part of the feed by-product. As part of a thermal recovery system, the evaporator is partially heated by vapour from the rotary dryer. The vapour, which condenses, contains some ethanol, so it is sent on to the stripper/distillation section for product recovery.

The Extractive Fermentation plant design, presented in Figure 3-2, was formulated with the aim of incorporating as few substantial changes as possible relative to the conventional plant. This would allow the flexibility of either building a grass-roots plant, or retrofitting an existing ethanol plant with Extractive Fermentation technology at minimal cost. In its preliminary design, this plant required only an extra centrifuge to separate the aqueous components and solids from the solvent, and a flash vaporization unit to remove ethanol from the solvent.

Both the conventional and Extractive Fermentation grass-roots plants were based on a capacity of 100 x 10^6 L/y of azeotropic ethanol. The feedstock was assumed to be essentially solids-free (5% wt inert solids) such as that produced by a corn wet-milling operation.

FIGURE 3-2

ETHANOL PRODUCTION USING EXTRACTIVE FERMENTATION


SOLVENT RECYCLE
CW
CENTRIFUGE
CW
FERMENTOR
FLASH
CW
ETHANOL STORAGE
FEED
CELL RECYCLE
CENTRIFUGE
AIR COMPRESSOR AND FILTER
ATMOSPHERIC DISTILLATION COLUMN
CW
CW
ROTARY DRYER
600 PSIG STEAM
STRIPPER
50 PSIG STEAM
YEAST STORAGE
EXHAUST STEAM
5 EFFECT STILLAGE EVAPO-RATOR
50 PSIG STEAM
50 PSIG STEAM

3.2 PROCESS^{TM} AND THE FERMENTOR MODEL

The conventional and Extractive Fermentation process flow diagrams were simulated using PROCESS^{TM} (Simulation Sciences Inc., Fullerton, California). This software package, which is primarily used in the chemical, petroleum, and synthetic fuels industries, features flexible calculation methods for unit operations, a large chemical component library, extensive thermodynamic properties prediction methods, optimization routines, and error checking systems. As well, PROCESS^{TM} allows users to incorporate their own FORTRAN routines for unique processing units, and thus permits extensive flexibility in formulating novel process designs.

PROCESS^{TM} contains numerous unit operation modules, each capable of simulating a particular piece of equipment. However, since Extractive Fermentation is a unique technology, PROCESS^{TM} does not contain a bioreactor module capable of simulating both conventional and Extractive Fermentation with cell recycle. To overcome this difficulty, a mathematical fermentor model, which is presented in Appendix A, was developed and incorporated into PROCESS^{TM} as a FORTRAN subroutine. This program uses the Newton-Raphson strategy for solving nonlinear systems of equations (Press, et al., 1986).

A similar model was written in TURBO PASCAL^{TM} for use on a personal computer. This model was used to predict process conditions under a variety of operating conditions and was used in experimental work with the Extractive Fermentation Process Demonstration Unit. Unlike the FORTRAN program, this program uses a bisection solution strategy.

3.3 MODEL ASSUMPTIONS

As with any model, various assumptions were made with the intention of modeling an actual plant with reasonable accuracy. As far as possible, these assumptions were based on actual values obtained from experimental work, or published data.

3.3.1 Equipment Operation

A full description of the assumptions used in equipment operation is given in Table 3-1.

Amongst these, the model describing the fermentor kinetics is of paramount importance. Several kinetic models have been proposed to describe ethanol production by fermentation (Aiba and Shoda, 1969; Zines and Rogers, 1970; Comberbach et al., 1986). The model described by the equation

$$\mu = \mu_{max} \left[\frac{S}{K_s+S}\right]\left[1 - \frac{P}{P_{max}}\right]^n$$

where μ - cell specific growth rate (h^{-1})
μ_{max} - maximum cell specific growth rate (h^{-1})
S - Aqueous substrate concentration (g/L)
K_s - Monod Constant (g/L)
P - Aqueous ethanol concentration (g/L)
P_{max} - Maximum aqueous ethanol concentration (g/L)
n - Toxic power coefficient

was chosen for the PROCESS™ simulations because it describes the observed phenomenon of zero cell growth and ethanol production at high ethanol concentrations and also the decreased cell activity at low substrate concentrations (Maiorella, et al., 1984a). Ethanol production was modeled by assuming that the formation of the product was directly related to cell production by a constant yield coefficient, as shown in Table 3-1. Depending on the value of the toxic power coefficient, the region approaching the maximum ethanol concentration as predicted by the kinetic equation may be inherently unstable. To prevent convergence problems, while still adequately describing fermentor kinetics, a value of 0.75 was arbitrarily chosen for the toxic power coefficient. Other equation parameters were based on experimental values and are presented in Table 3-1.

TABLE 3-1

EQUIPMENT OPERATION ASSUMPTIONS

FERMENTOR:	
Cell Concentration	75 g/L
Maximum ethanol concentration (P_{max})	87.5 g/L
Kinetic model	$\mu=\mu_{max}\cdot S/(K_s+S)\cdot(1-P/P_m)^n$
Toxic power coefficient (n)	0.75
Specific growth rate (μ_{max})	0.328 h^{-1}
Monod constant (K_s)	2.4 g/L
Ethanol yield coefficient	7.55 g ethanol/g cells
Maximum fermentor volume	200 m^3
Head room	10 % of aqueous volume
Solvent room	10 % of aqueous volume
Overall heat transfer coefficient	1135 $W/m^2\cdot{}^\circ C$
Temp. cooling water in	10 °C
Temp. cooling water out	25 °C
Fermentor temperature	30 °C
Fermentor agitator power	0.5 kW/m^3
Solids (unfermentable) conc. in feed	5 % (wt)
Solvent distribution coefficient	0.25 g/g
Extent of equilibrium	100 %
CENTRIFUGE:	
Power requirement	0.0011 kW/kg flow
Mass fraction cells in centrifuge concentrate stream	0.2
EVAPORATOR:	
Concentration of solids in evaporator	30 % (wt)
Overall heat transfer coefficient	2271 $W/m^2\cdot{}^\circ C$
Boiling point rise from fermentables	Not taken into account
Heat source	50 psig steam
Re-used exhaust steam	25 % of total exhaust stream
ROTARY DRYER:	
Power requirement	0.0059 kW/kg evaporate flow
Final moisture content of solids	8 % (wt)
Heat source	600 psig steam
DISTILLATION COLUMN:	
Number of theoretical trays	40 + 1 reboiler + 1 total condenser
Operating pressure	Atmospheric pressure
Ethanol concentration overhead	93.5 % (wt)
Ethanol recovery	99.5 % (wt)
STRIPPER:	
Number of theoretical trays	15 + 1 reboiler
Ethanol concentration overhead	approximately 44 % (wt)
Operating pressure	Atmospheric pressure
Ethanol concentration in bottoms	0 % (wt)

3.3.2 Raw Materials And Utilities

The raw materials and utilities costs, presented in Table 3-2, were based on values obtained from suppliers, from ethanol producers, or from ethanol plant construction companies. Both the dried yeast product and solid debris can be sold as animal feed, and thus represent a credit.

TABLE 3-2

PRICES FOR RAW MATERIALS AND UTILITIES

Material	Cost
Fermentables	0.15 \$/kg
Solvent	4.75 \$/kg
Dried yeast (credit)	0.537 \$/kg
Solid debris (credit)	0.195 \$/kg
Atmospheric steam	2.58 \$/t
LP steam (50 psig)	13.36 \$/t
HP steam (600 psig)	14.26 \$/t
Cooling water	0.312 $\$/m^3$
Electricity:	\$0.095/kWh for <250 kWh/month \$0.0584/kWh for <12250 kWh/month \$0.0417/kWh for <2080000 kWh/month \$0.025/kWh for >2080000 kWh/month

All prices and costs used in the PROCESS™ simulations were based on January, 1989 Canadian dollars, with a Marshall and Swift Index of 900. The exchange rate between Canadian and U.S. dollars was assumed to be \$1.00 Can = \$0.80 U.S.

3.3.3 Equipment Costing Correlations

The purchase prices of the major items of equipment were obtained by equipment costing correlations, according to standard engineering practice (Woods, 1975; Peters and Timmerhaus, 1980). These correlations, which are presented in Table 3-3, were initially based on data from Maiorella (Maiorella, *et al.*, 1984a) and were assumed to follow the general cost correlation:

$$\text{Cost} = (\text{Reference Cost})\left[\frac{\text{Capacity}}{\text{Reference Capacity}}\right]^n$$

For example, according to Maiorella, an air filter with a capacity of 818 kg air/h cost \$310 U.S. in mid-1981 (MSI=717), while a filter with a capacity of 1200 kg air/h cost \$400 U.S. in the same year. Using the general cost correlation:

$$\$400 = \$310 \left[\frac{1200}{818} \right]^{n}$$

$$n \cong 0.67$$

From this, the cost correlation in Table 3-3 was developed, assuming an exchange rate of \$1.00 CAN = \$0.80 U.S.:

$$\text{COST} = (310) \left[\frac{1}{0.80} \right] \left[\frac{\text{MSI}}{717} \right] \left[\frac{\text{CAPACITY}}{818} \right]^{0.67}$$

$$\text{COST} = (0.007)(\text{MSI})(\text{CAPACITY})^{0.67}$$

Each correlation was compared, if possible, to correlations from other sources (Woods, 1975; Peters and Timmerhaus, 1980; Ulrich, 1984) to ensure its accuracy and applicability to ethanol plant operation. For example, a storage tank with a 5.0×10^{6} L capacity would cost (in 1989 Canadian dollars) \$303,000 according to Table 3-3, compared to \$250,000 (Peters and Timmerhaus), and \$182,000 (Ulrich).

TABLE 3-3

EQUIPMENT COST CORRELATIONS

Item	Unit Cost Correlation	Capacity Units
Storage Tank	MSI x 0.205 x $\text{Capacity}^{0.48}$	L
Fermentor	MSI x 0.204 x $\text{Capacity}^{0.55}$	L
Fermentor Agitator	MSI x 6.236 x $\text{Capacity}^{0.67}$	kW
Fermentor Cooler	MSI x 2.372 x $\text{Capacity}^{0.32}$	m^2
Air Filter	MSI x 0.007 x $\text{Capacity}^{0.67}$	kg gas/h
Air Compressor	MSI x 8.782 x $\text{Capacity}^{0.32}$	kg gas/h
Centrifuge	MSI x 0.022 x $\text{Capacity}^{0.86}$	kg feed/h
Rotary Dryer	MSI x 19.63 x $\text{Capacity}^{0.47}$	kg evaporate/h
Stripper/Distillation	MSI x 109.9 x $\text{Capacity}^{0.14}$	kg water/h
Stillage Evaporator	MSI x 4.752 x $\text{Capacity}^{0.55}$	kg feed/h
Flash Unit	MSI x 0.222 x $\text{Capacity}^{0.55}$	L

MSI = Marshall and Stevens Index = 900 for 1989. All costs in \$CAN.

3.3.4 Grass-Roots Development and Plant Operation

Other assumptions were made regarding the actual construction of the plant and the fixed costs involved in its subsequent operation. Table 3-4 lists the factors used to calculate the fixed capital investment from the total delivered equipment cost (Perry and Green, 1984). As shown, the fixed capital investment is 4.04 x the total delivered equipment cost. Other assumptions concerning plant operation and the derivation of fixed production costs are presented in Table 3-5.

TABLE 3-4

FACTORS TO CONVERT DELIVERED EQUIPMENT COSTS TO FIXED CAPITAL INVESTMENT

DETAILS		CONVERSION FACTORS FOR A GRASS-ROOTS FACILITY
Delivered Equipment (not including pumps)		1.00
Installation (including foundations)		1.04
Piping		0.33
Electrical		0.09
Instrumentation		0.13
Battery Limits Building and Services (indoor facility)		0.45
Land (includes pumps, land, yard improvements)		0.06
	Total Physical Plant	3.10
Engineering		0.41
	Direct Plant Cost	3.51
Contractor Fees		0.17
Contingency		0.36
	Total Fixed Capital	4.04 x Delivered Cost

TABLE 3-5

PLANT INVESTMENT AND OPERATION ASSUMPTIONS

Operating labour	6 people/shift
Wages	18 $/person·hour
Supervisory/clerical	15 % of operating labour
Maintenance/repairs	4 % of fixed investment capital
Operating supplies	15 % of maintenance/repairs
Laboratory expenses	15 % of operating labour
Overhead	60 % of operating, maintenance, and supervisory labour
Property tax	2 % of fixed investment capital
Insurance	1 % of fixed investment capital
Administration	25 % of operating labour
Distribution	10 % of total manufact. expense
Plant life	25 years
Salvage value	0 $
Return on investment (after tax)	15 % of total capital investment
MS index	900
Total delivered cost	1.15 x total purchased equip. cost
Total fixed capital	4.04 x total delivered cost
Total working capital	10 % of total investment
Inflation	5 %/year
Income tax	50 % of net profit before tax
Grass-roots plant construction period	2 years
Total Working Capital	INCLUDES COSTS FOR: a. 1 month's stock of raw materials and supplies at delivered prices b. 1 month's manufacturing costs for finished product in stock c. Accounts receivable (1 month's production costs, salaries, and wages) and taxes payable
Production period	330 days
Plant capacity	100 x 10^6 L/y azeotropic ethanol
Depreciation	Straight line

3.4 SIMULATION RESULTS

3.4.1 Conventional Plant Simulation

Optimization of the conventional ethanol plant involved manipulating two input variables: the concentration of fermentables in the fermentor feed stream and the fermentor feed dilution rate. The dilution rate is a normalized feed flowrate and is defined as the volumetric feed flowrate divided by the aqueous fermentor volume. It is equivalent to the reciprocal of the aqueous retention time in the fermentor. For each feed concentration it can be expected that an optimum (lowest cost) situation exists. As dilution rate is increased (retention time decreased) the ethanol level in the fermentor is reduced, resulting in higher reaction rate and thus reduced reactor size. However, the residual fermentables concentration increases and greater costs are incurred in distilling the lower ethanol concentration to the azeotrope. These competing situations will produce a "lowest cost" case for each fermentables concentration from which a global optimum condition can be found.

A feed concentration of 18% (wt) fermentables and a dilution rate of 0.20 h^{-1} was found to provide the lowest ethanol production cost, as shown in Figure 3-3. Under these conditions, the fermentor would operate at 82.0 g/L ethanol and 5.5 g/L residual fermentables, with a total productivity of 16.3 g/L·h. Stream flows for the conventional plant can be found in Appendix B. A complete economic analysis is presented in Table 3-6, revealing a total capital investment of $31,043,000, and an ethanol selling price of 45.06 ¢/L for a 15% return on investment.

FIGURE 3-3

OPTIMUM OPERATING CONDITIONS
Conventional Fermentation (Green Fields)

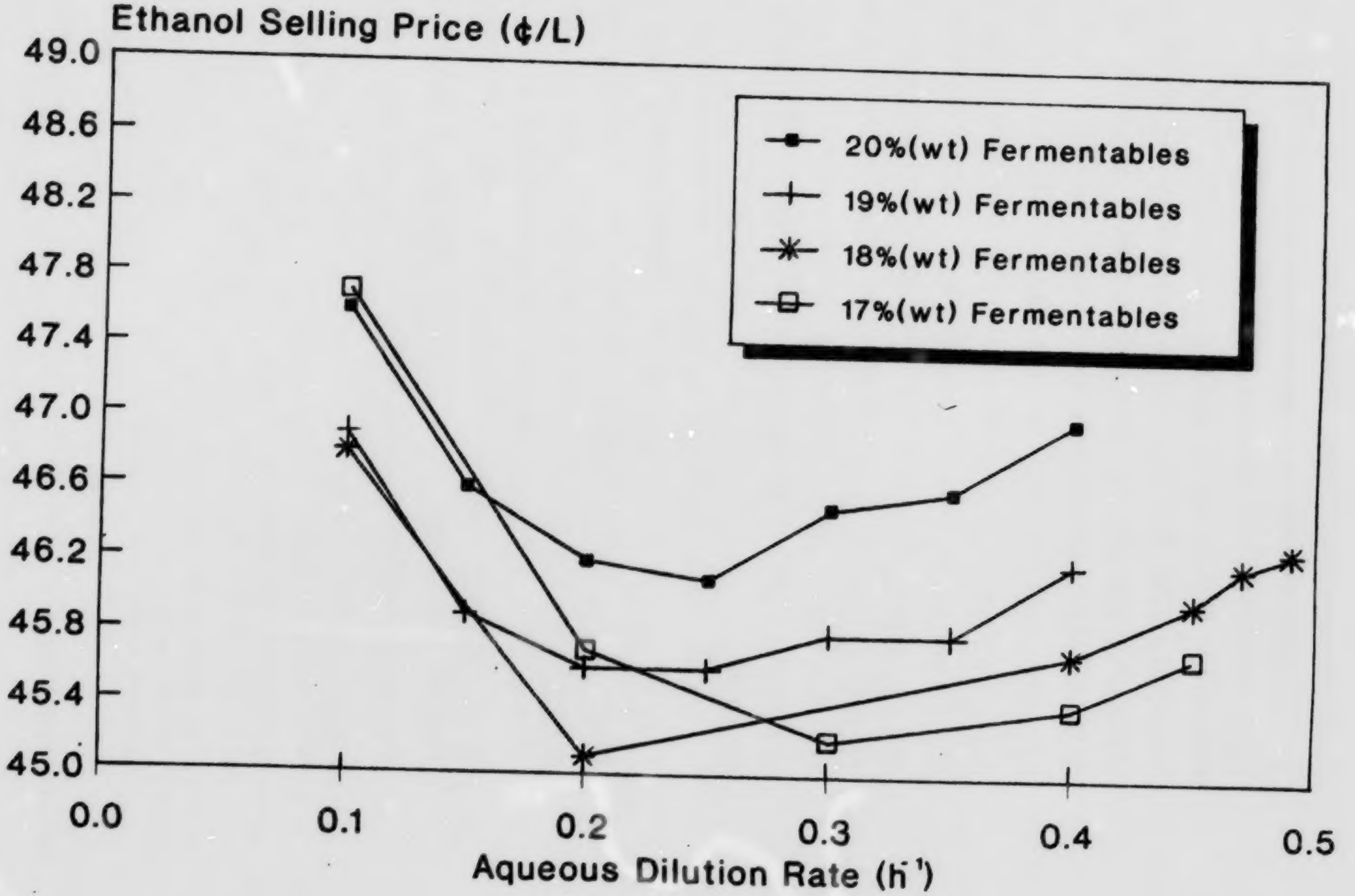


TABLE 3-6

Economic Results for Conventional Fermentation, Grass Roots (98.5×10^6 L/y of Azeotropic Ethanol)

COSTS		
Purchased Equipment:		
Storage	$445,000	
Fermentation	$1,669,000	
Dryer	$1,049,000	
Stripper	$504,000	
Evaporator	$2,407,000	
Total Purchased Equip.	$6,074,000	
Total Delivered Equip.	$6,985,000	
Total Fixed Capital Invest.	$28,221,000	
Total Working Capital	$2,822,000	
Total Capital Investment	$31,043,000	
MANUFACTURING EXPENSES		
Direct Expenses:	$/y	¢/L
Raw Materials	$26,618,000	27.01
Utilities;		
50 psig steam	$9,325,000	9.46
600 psig steam	$1,035,000	1.05
Process and cooling water	$706,000	0.72
Electricity	$491,000	0.50
Operating Labour	$855,000	0.87
Supervisory Labour	$128,000	0.13
Maintenance	$1,129,000	1.15
Operating Supplies	$169,000	0.17
Laboratory Expenses	$128,000	0.13
Indirect Expenses:		
Overhead	$1,268,000	1.29
Property Tax	$564,000	0.57
Insurance	$282,000	0.29
General Expenses:		
Administration	$214,000	0.22
Distribution/marketing	$4,270,000	4.33
Total Production Expense	$47,183,000	47.88
Net Profit After Tax	$4,656,000	4.73
Income Tax	$4,656,000	4.73
Credits	($13,337,000)	-13.54
Depreciation	$1,242,000	1.26
Total Production Cost	$44,400,000	45.06

3.4.2 Extractive Fermentation Plant Simulation

The optimization of the Extractive Fermentation plant required manipulation of the fermentor feed dilution rate (D) and, additionally, the solvent dilution rate (Ds), which is defined as the solvent volumetric flowrate divided by the aqueous fermentor volume. The solvent is ADOL® 85 NF (Sherex Chemical Co., Dublin, OH) which is a mixture of straight-chain, mono-unsaturated primary alcohols (69% oleyl alcohol) and which is used commercially in pharmaceutical and cosmetic preparations. The fermentables concentration in the feed was arbitrarily fixed at 41.5% by weight (500 g/L) because this level of fermentables can be achieved by wet milling operations and because Extractive Fermentation can readily and effectively operate at this feed concentration (Kollerup and Daugulis, 1987). The optimization procedure involved a thee-dimensional grid search to find the lowest production cost. The results of this search, presented in Figure 3-4, show a minimum cost trough, indicating not simply one set of optimal operating conditions, but several. For example, the optimum ethanol production cost of 38.69 ¢/L was obtained at D = 0.15 h^{-1},Ds = 1.25 h^{-1}, and at D = 0.18 h^{-1}, Ds = 1.50 h^{-1}. The former set of operating conditions was chosen as the basis for the grass-roots economic analysis. Under these conditions, PROCESS™ predicted the fermentor ethanol and residual fermentables concentrations to be 75.3 g/L and 7.5 g/L, respectively. Total ethanol productivity was predicted to be 32.0 g/L·h. A complete material balance for this simulation is presented in Appendix B.

A full economic analysis of the optimal grass-roots Extractive Fermentation plant is shown in Table 3-7. In this analysis, multiplier factors do not apply to the solvent inventory because it is not a piece of equipment that has to be installed. Instead, the cost of 250,000 kg of solvent at $4.75/kg is added directly to the total capital investment. The analysis indicates a total capital investment of $27,964,000 and an ethanol selling price of 38.69 ¢/L for a 15% return on investment. Compared to the conventional plant, this represents an additional revenue of $6,250,000/y. Alternatively, selling the ethanol produced by the Extractive Fermentation plant at the conventional plant price would provide a 26% return on investment (ROI), almost double the conventional plant ROI.

One of the largest cost savings with an Extractive Fermentation plant is in the area of energy. From Tables 3-6 and 3-7, the Extractive Fermentation

FIGURE 3-4

Optimum Operating Conditions
For Extractive Fermentation (green fields)
Fermentables in Feed = 500 g/l (41.5 wt. %)

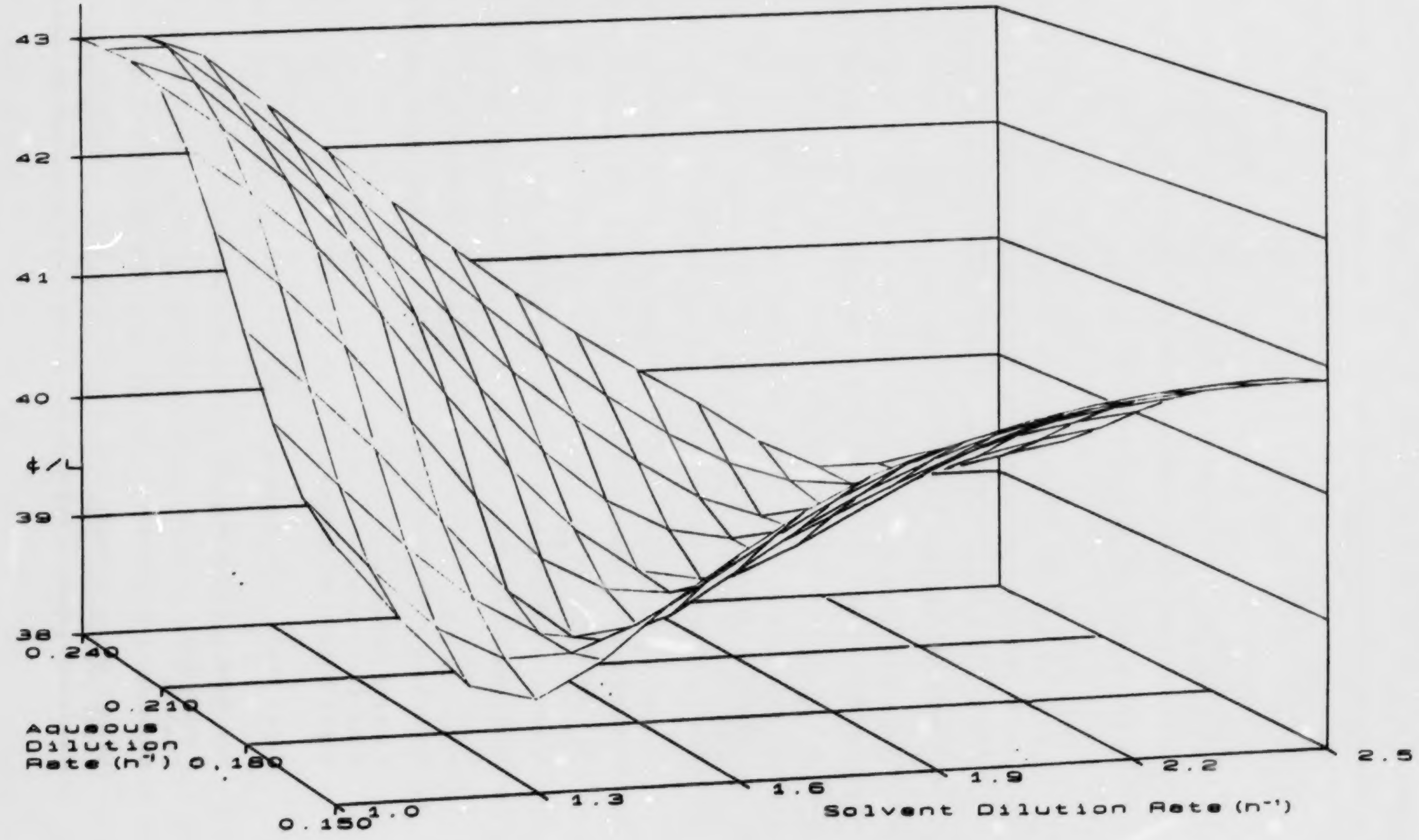


TABLE 3-7

Economic Results for Extractive Fermentation, Grass Roots
(98.2x10^6 L/y of Azeotropic Ethanol)

COSTS

Purchased Equipment:	
Storage	$495,000
Fermentation	$777,000
Dryer	$1,029,000
Stripper	$426,000
Evaporator	$1,240,000
Flash Unit	$156,000
Solvent Centrifuge	$1,064,000
Total Purchased Equip.	$5,187,000
Total Delivered Equip.	$5,965,000
Total Fixed Capital Invest.	$24,099,000
Total Working Capital	$2,410,000
Total Capital Investment	$27,964,000

MANUFACTURING EXPENSES

Direct Expenses:	$/y	¢/L
Raw Materials	$26,325,000	26.82
Utilities;		
50 psig steam	$4,317,000	4.40
600 psig steam	$1,001,000	1.02
Process and cooling water	$1,206,000	1.23
Electricity	$341,000	0.35
Operating Labour	$855,000	0.87
Supervisory Labour	$128,000	0.13
Maintenance	$964,000	0.98
Operating Supplies	$145,000	0.15
Laboratory Expenses	$128,000	0.13
Indirect Expenses:		
Overhead	$1,169,000	1.19
Property Tax	$482,000	0.49
Insurance	$241,000	0.25
General Expenses:		
Administration	$214,000	0.22
Distribution/marketing	$3,730,000	3.80
Total Production Expense	$41,246,000	42.02
Net Profit After Tax	$4,195,000	4.27
Income Tax	$4,195,000	4.27
Credits	($12,775,000)	-13.02
Depreciation	$1,119,000	1.14
Total Production Cost	$37,979,000	38.69

plant requires less than half the amount of low pressure steam required by the conventional plant. This is due to the reduced amount of downstream water processing that is needed for Extractive Fermentation, particularly in the stillage evaporator. For this unit alone, the steam cost in the conventional plant would be $5,618,000/y compared to $1,029,000/y in the Extractive Fermentation plant.

The distillation requirements of the conventional and Extractive Fermentation plants were investigated to determine if a redesigned distillation column for Extractive Fermentation would provide any additional cost benefits (McLellan, 1989). The feed to the distillation column for the Extractive Fermentation plant has a higher percentage of ethanol than the same stream in the conventional plant (69% wt compared to 45% wt). Thus it would seem intuitive that Extractive Fermentation would require a distillation column with fewer stages and lower condenser and reboiler duties. However, a rigorous analysis concluded that condenser duties would be the same because the reflux ratios and production rates of distillate for both columns must be the same. Also, the maximum difference in reboiler duties between the two cases constitutes about 2% of the typical reboiler duty; thus, the condensers and reboilers for both columns would be the same design. As well, the number of stages for the extractive plant could be reduced by a maximum of 10 - 11 stages, resulting in a small capital cost saving. However, it was decided that this saving would be insignificant compared to the overall cost savings of Extractive Fermentation, and so the same number of stages was assumed for both cases. It was also determined that any cost savings from using a vacuum distillation column would apply equally to both the conventional and extractive cases. Thus, the distillation columns used for both plant simulations were identical in design.

3.4.3 Retrofit Simulation

Since the conventional and Extractive Fermentation plants are almost identical in design, it should be possible to retrofit a conventional plant with Extractive Fermentation technology at a substantial cost savings over the grass-roots plant. This concept was investigated using PROCESS™. None of the existing equipment modules in the PROCESS™ conventional plant simulation were altered for the retrofit simulations. However, the addition of Extractive Fermentation technology, required incorporating a solvent centrifuge and a flash unit into the original conventional plant simulation program. Also, unlike the Extractive Fermentation grass-roots simulations, the retrofit simulations did not place restrictions on the amount of ethanol produced. That is, ethanol production was allowed to fall where it may, instead of being specified as 100 x 10^6 L/y. Since Extractive Fermentation increases the amounts of both ethanol and yeast produced, an extra rotary dryer unit and extra storage facilities for ethanol and yeast, as well as for solvent, had to be included in the retrofit cost analysis. It is important to note that the distillation module used in PROCESS™ was formulated to accommodate different stream conditions simply by changing operating parameters, such as reflux ratio, without changing the column model. Thus, the column used in the conventional simulations was physically identical to that used in the retrofit scenarios, implying that in an actual retrofitted plant, the distillation column would not have to be physically altered in any fashion.

For the retrofitted plant simulation the only additional capital investment was for the delivered cost of the retrofit equipment (centrifuge, flash unit, rotary dryer, extra storage) and solvent. The multiplication factors presented in Table 3-4 were not used to obtain total working capital because they were designed for grass-roots analysis. For a retrofitted plant, factors for items such as land and buildings would not apply, while those for piping and electrical would be greatly reduced (since the existing facility would already have extensive piping and electrical services in place) and would have to be arbitrarily assigned. Also, any modest price increase that would be realized by applying the appropriate multiplication factors would be mitigated, since it was assumed that the retrofit equipment is depreciated over only 10 years, and that its salvage value is zero.

Raw materials and utilities were those needed for operation of the retrofitted plant. Fixed expenses, such as advertising, laboratory expenses, and the like, were assumed to be identical to those in the conventional plant. Depreciation on the new equipment was straight line over 10 years since it was assumed that the retrofitted plant would have 10 years left in its lifespan. Depreciation on the existing equipment was assumed to be the same as the conventional plant. Solvent was not depreciated. The same profit for the conventional plant was maintained for the retrofitted plant, with an additional 15% return on the new capital investment. The sum of these two, formed the new profit margin which was maintained for the retrofitted plant. Income tax remained at 50% of the net profit before tax. A payback period was calculated by assuming that the extra revenue generated from producing ethanol at a lower cost would be used to pay off the retrofit capital investment.

Three situations were investigated. For the first two, operating conditions were based on the optimal conditions predicted in Figure 3-4. Thus, one simulation used operating conditions of $D = 0.15\ h^{-1}$ and $Ds = 1.25\ h^{-1}$ (simulation RETRO1), while the second used $D = 0.18\ h^{-1}$ and $Ds = 1.50\ h^{-1}$ (simulation RETRO2). As shown in Tables 3-8 and 3-9, these provided ethanol selling prices of 32.70 ¢/L and 31.01 ¢/L, respectively, compared to 45.06 ¢/L for the conventional plant. It is also obvious that retrofitting a conventional plant with Extractive Fermentation technology increases plant capacity. The conventional plant, which produced 98.5×10^6 L of ethanol per year, increased its capacity to 176.6×10^6 L/y with RETRO1 and 209.7×10^6 L/y with RETRO2. Since larger amounts of yeast were also produced, larger yeast credits were realized with the retrofitted plants. The short payback period also makes the retrofit scenario financially attractive.

TABLE 3-8

Economic Analysis for Simulation RETRO1

	$ CAN
Capital Costs:	
Rotary Dryer	0.893×10^6
Centrifuge/Flash Units	1.979×10^6
Storage	0.416×10^6
Solvent	1.188×10^6
Total Purchased Cost	4.476×10^6
Total Delivered Cost	5.147×10^6
Production Costs:	
Raw Materials and Utilities	59.734×10^6
Fixed Expenses	9.008×10^6
Depreciation (solvent not included)	1.620×10^6
Cost Summary:	
Production	70.362×10^6
Profit	4.656×10^6
Add ROI	0.772×10^6
Tax	5.428×10^6
Credits	-23.474×10^6
Total	57.744×10^6
Plant Capacity (L/y)	176.607×10^6
Ethanol Selling Price (¢/L)	32.70
Conv. Selling Price (¢/L)	45.06
Payback Period	2.6 months

TABLE 3-9

Economic Analysis for Simulation RETRO2

Capital Costs:	$ CAN
Rotary Dryer	1.015×10^6
Centrifuge/Flash Units	2.301×10^6
Storage	0.480×10^6
Solvent	1.188×10^6
Total Purchased Cost	4.984×10^6
Total Delivered Cost	5.732×10^6
Production Costs	
Raw Materials and Utilities	71.561×10^6
Fixed Expenses	9.008×10^6
Depreciation (solvent not included)	1.679×10^6
Cost Summary:	
Production	82.248×10^6
Profit	4.656×10^6
Add ROI	0.860×10^6
Tax	5.516×10^6
Credits	-28.269×10^6
Total	65.011×10^6
Plant Capacity (L/y)	209.672×10^6
Ethanol Selling Price (¢/L)	31.01
Conv. Selling Price (¢/L)	45.06
Payback Period	2.1 months

The simulations RETRO1 and RETRO2 still did not provide the lowest production cost possible because of inefficient equipment operation. Since retrofitting an existing plant with Extractive Fermentation technology drastically reduces water usage, most of the downstream equipment in the first two retrofit simulations was oversized, and thus operated inefficiently. To overcome this difficulty, a third simulation (**RETRONEW**) was performed in which the fermentor aqueous dilution rate was approximately the same (0.22 h^{-1}) as the conventional plant. This resulted in "on-spec" stream flows to the major pieces of equipment. With the aqueous dilution rate fixed, the solvent dilution rate was chosen as 2.0 h^{-1} based on Figure 3-4.

A comparison between RETRONEW and the conventional plant simulation is presented in Table 3-10. As shown, this retrofitted plant requires a total capital investment of only \$6,768,000 and an ethanol selling price of only 29.44 ¢/L for a 15% return on investment. This corresponds to increased revenue of \$24,421,000/y over the conventional plant. Also, it can be seen that the large decrease in selling price is not so much due to energy savings, as was the case for the grass-roots Extractive Fermentation plant, but is due to the large increase in plant capacity over the conventional plant that occurs by simply adding Extractive Fermentation technology. As well, a much larger yeast credit is realized because of increased cell production. A short payback period of 1.8 months makes this alternative particularly attractive.

TABLE 3-10

Economic Comparison of RETRONEW to Conventional Plant
(Conventional Production - 98,539,000 L/y Azeotropic Ethanol)
(Retrofitted Production - 254,882,000 L/y Azeotropic Ethanol)

COSTS ($CAN)	Conventional Plant		Retrofitted Plant	
Purchased Equipment:				
Storage	$445,000		$550,000	
Fermentation	$1,669,000			
Dryer	$1,049,000		$1,230,000	
Stripper	$504,000			
Evaporator	$2,407,000			
Centrifuge/Flash			$2,917,000	
Solvent			$1,188,000	
Total Purchased Equip.	$6,074,000		$5,885,000	
Total Delivered Equip.	$6,985,000		$6,768,000	
Total Fixed Capital Invest.	$28,221,000			
Total Working Capital	$2,822,000			
Total Capital Investment	$31,043,000		$6,768,000	
MANUFACTURING EXPENSES				
Direct Expenses:	$/y	¢/L	$/y	¢/L
Raw Materials	$26,618,000	27.01	$68,865,000	27.02
Utilities;				
50 psig steam	$9,325,000	9.46	$11,581,000	4.54
600 psig steam	$1,035,000	1.05	$2,520,000	0.99
Process and cooling water	$706,000	0.72	$3,280,000	1.29
Electricity	$491,000	0.50	$861,000	0.34
Operating Labour	$855,000	0.87	$855,000	0.34
Supervisory Labour	$128,000	0.13	$128,000	0.05
Maintenance	$1,129,000	1.15	$1,129,000	0.44
Operating Supplies	$169,000	0.17	$169,000	0.07
Laboratory Expenses	$128,000	0.13	$128,000	0.05
Indirect Expenses:				
Overhead	$1,268,000	1.29	$1,268,000	0.50
Property Tax	$564,000	0.57	$564,000	0.22
Insurance	$282,000	0.29	$282,000	0.11
General Expenses:				
Administration	$214,000	0.22	$214,000	0.08
Distribution/marketing	$4,270,000	4.33	$4,270,000	1.68
Total Production Expense	$47,183,000	47.88	$96,115,000	37.71
Net Profit After Tax	$4,656,490	4.73	$4,656,000	1.83
Income Tax	$4,656,490	4.73	$5,671,000	2.22
Credits	($13,337,300)	-13.54	($34,194,000)	-13.42
Depreciation	$1,241,732	1.26	$1,782,000	0.70
Additional ROI			$1,015,000	0.40
Total Production Cost	$44,399,944	45.06	$75,045,000	29.44

3.5 SUMMARY OF SIMULATION WORK

Ethanol production by Extractive Fermentation is an economically attractive alternative to conventional fermentation. Economic optimization of a computer-simulated grass-roots ethanol plant, which used conventional ethanol production technology and had a production capacity of 100 x 10^6 L/y of azeotropic ethanol, yielded an ethanol selling price of 45.06 ¢/L for a 15% return on investment. Optimization of a similar grass-roots plant, which used Extractive Fermentation technology and with the same production capacity, yielded a lower selling price of 38.69 ¢/L for a 15% return on investment.

The similar design of the conventional and Extractive Fermentation plants allows low-cost retrofitting of a conventional plant with Extractive Fermentation technology for even greater cost savings. Simulation of one retrofitted plant yielded the lowest ethanol selling price of 29.44 ¢/L.

4.0 EXPERIMENTAL WORK

The purpose of the experimental work was to verify that the operating conditions predicted though computer simulation could be achieved in practice. This was accomplished with an Extractive Fermentation Process Demonstration Unit (PDU) which was upgraded with more sophisticated equipment and process control.

4.1 EXTRACTIVE FERMENTATION PROCESS DEMONSTRATION UNIT

A flow diagram of the Extractive Fermentation test unit currently used at Queen's University is shown in Figure 4-1. For clarity, a photograph of the system is shown in Figure 4-2. During conventional fermentation, medium is fed from a 16 L medium carboy to a 7 L (total volume) fermentor by a peristaltic pump using size 14 Tygon® tubing. The fermentor broth is withdrawn from the fermentor by a diaphragm pump. The fermentor volume is controlled at about 4.8 L by withdrawing liquid at a higher rate than the feed rate from a fixed level. The centrifuge concentrates the cells which accumulate inside the centrifuge bowl and are discharged periodically as determined by an automatic timer. A portion is returned to the fermentor for cell recycle. A dilute aqueous stream also exits the centrifuge and collects in the aqueous carboy.

FIGURE 4-1

Flow Diagram Of Extractive Fermentation Process Demonstration Unit

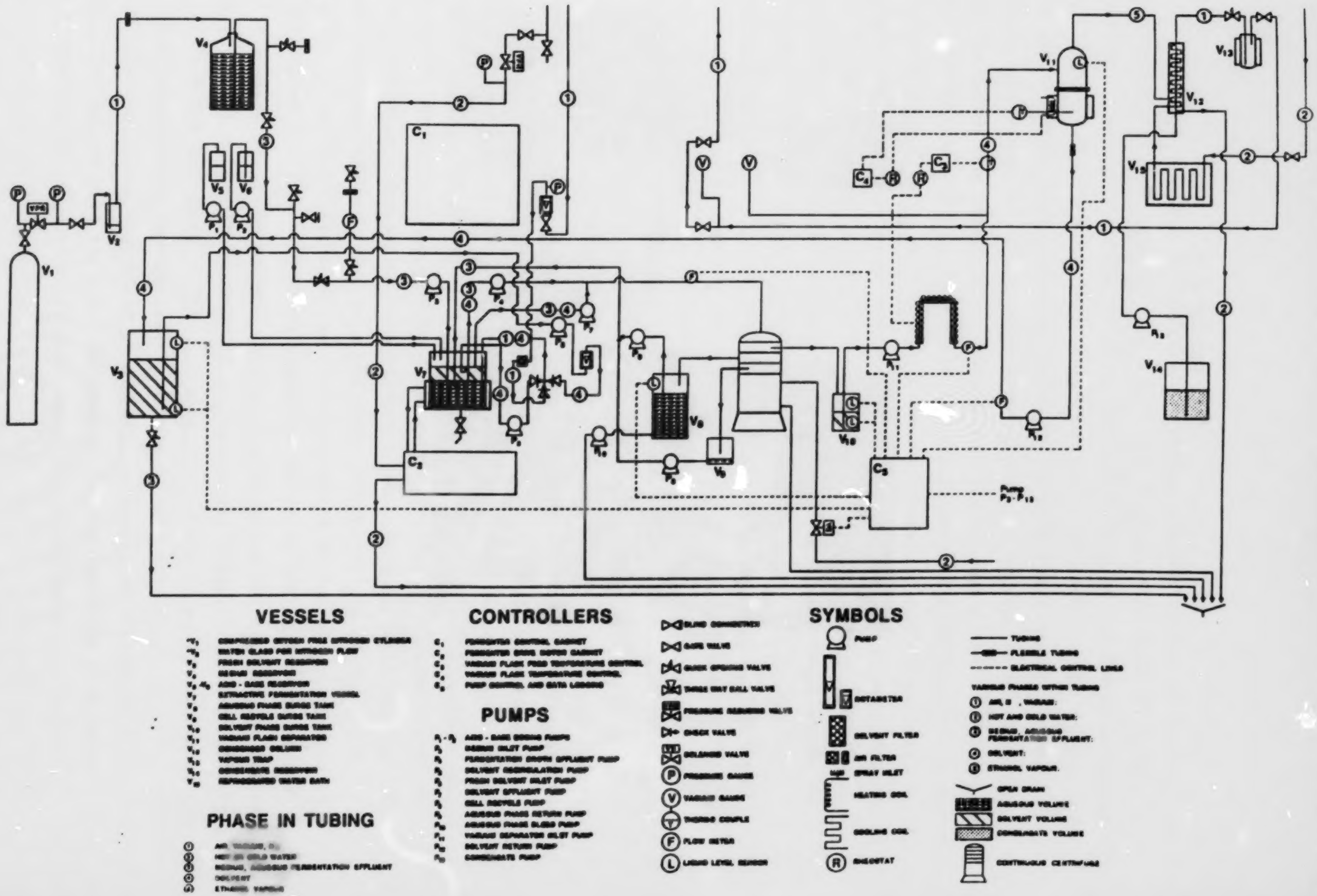
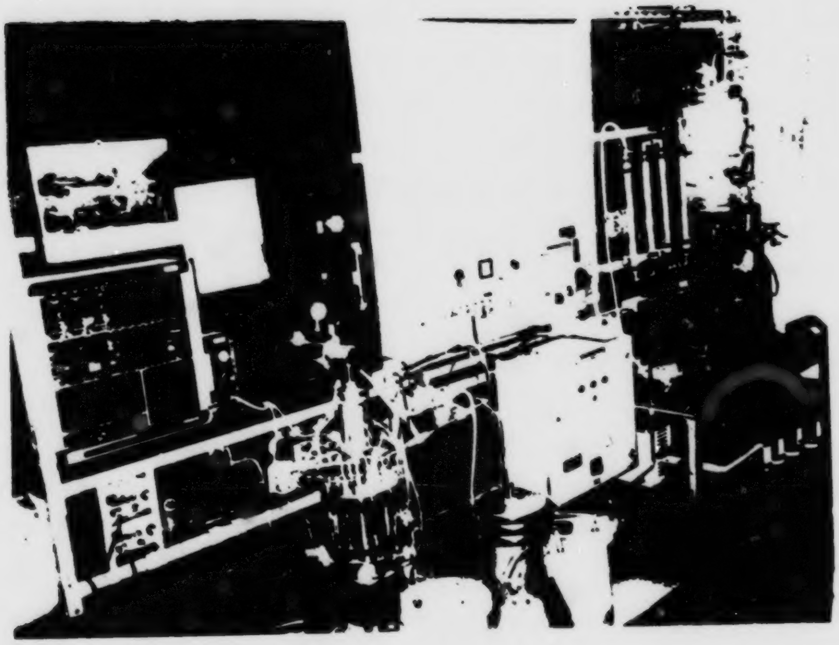

V1
V2
V3
V4
V5
V6
V7
V8
V9
V10
V11
V12
V13
V14
V15
C1
C2
C3
C4
C5
P1
P2
P3
P4
P5
P6
P7
P8
P9
P10
P11
P12
R1
R2
R3
VPS
Pump P3 - P13
VESSELS
V1 COMPRESSED OXYGEN FREE NITROGEN CYLINDER
V2 WATER GLASS FOR NITROGEN FLOW
V3 FRESH SOLVENT RESERVOIR
V4 MEDIUM RESERVOIR
V5 - V6 ACID - BASE RECEIVER
V7 EXTRACTIVE FERMENTATION VESSEL
V8 AQUEOUS PHASE SURGE TANK
V9 CELL RECYCLE SURGE TANK
V10 SOLVENT PHASE SURGE TANK
V11 VACUUM FLASH SEPARATOR
V12 CONDENSER COLUMN
V13 VAPOUR TRAP
V14 CONDENSATE RECEIVER
V15 REFRIGERATED WATER BATH
CONTROLLERS
C1 FERMENTOR CONTROL CABINET
C2 FERMENTOR BASE METER CABINET
C3 VACUUM FLASH FEED TEMPERATURE CONTROL
C4 VACUUM FLASK TEMPERATURE CONTROL
C5 PUMP CONTROL AND DATA LOGGING
PUMPS
P1 - P2 ACID - BASE DOSING PUMPS
P3 MEDIUM INLET PUMP
P4 FERMENTATION BROTH EFFLUENT PUMP
P5 SOLVENT RECIRCULATION PUMP
P6 FRESH SOLVENT INLET PUMP
P7 SOLVENT EFFLUENT PUMP
P8 CELL RECYCLE PUMP
P9 AQUEOUS PHASE RETURN PUMP
P10 AQUEOUS PHASE BLEED PUMP
P11 VACUUM SEPARATOR INLET PUMP
P12 SOLVENT RETURN PUMP
P13 CONDENSATE PUMP
PHASE IN TUBING
① AIR, VACUUM, N2
② HOT OR COLD WATER
③ MEDIUM, AQUEOUS FERMENTATION EFFLUENT
④ SOLVENT
⑤ ETHANOL VAPOUR
SYMBOLS
BLIND CONNECTION
GATE VALVE
QUICK OPENING VALVE
THREE WAY BALL VALVE
PRESSURE REDUCING VALVE
CHECK VALVE
SOLENOID VALVE
PRESSURE GAUGE
VACUUM GAUGE
THERMO COUPLE
FLOW METER
LIQUID LEVEL SENSOR
PUMP
ROTAMETER
SOLVENT FILTER
AIR FILTER
SPRAY INLET
HEATING COIL
COOLING COIL
RHEOSTAT
TUBING
FLEXIBLE TUBING
ELECTRICAL CONTROL LINES
VARIOUS PHASES WITHIN TUBING
① AIR, N2, VACUUM;
② HOT AND COLD WATER;
③ MEDIUM, AQUEOUS FERMENTATION EFFLUENT;
④ SOLVENT;
⑤ ETHANOL VAPOUR.
OPEN DRAIN
AQUEOUS VOLUME
SOLVENT VOLUME
CONDENSATE VOLUME
CONTINUOUS CENTRIFUGE

FIGURE 4-2

Photograph of Extractive Fermentation PDU



During Extractive Fermentation operation, solvent is fed to the fermentor from a 60 L solvent reservoir by a peristaltic pump using size 17 Tygon® tubing. The solvent (ADOL® 85 NF) enters the bottom of the fermentor though a ring sparger and, being less dense than water, collects at the top where it is withdrawn at a point somewhat above the aqueous phase take-off; thus, a layer of solvent with a volume of about 10% of the aqueous volume is formed at the top of the fermentor and floats upon the aqueous phase. In most cases it is not necessary to withdraw the aqueous phase in a separate stream, for two reasons. First, there is a significant volumetric difference between the aqueous inlet flowrate and the aqueous outlet flowrate during Extractive Fermentation due to the high glucose conversion and the removal of ethanol from the fermentation broth by the solvent. Secondly, the large amount of CO_2 produced tends to carry enough of the aqueous broth into the solvent phase such that the aqueous outlet volumetric flowrate leaving with the solvent phase often exceeds the aqueous inlet flowrate. Because of this the fermentor aqueous volume is controlled by returning a portion of the fermentation broth from the centrifuge aqueous carboy back to the fermentor.

The aqueous phase and cells are separated from the solvent in the centrifuge. The solvent collects in a 25 L solvent surge tank, while the aqueous phase and cells follow the same paths as for conventional operation. The solvent is pumped to a heating section which consists of electrical heating tape on 0.5 inch stainless steel tubing. The heating tapes are regulated by OMEGA temperature controllers connected to a thermocouple which measures the solvent temperature as it enters the flash. Inside the flash unit, which is under vacuum, the solvent flows over glass-bead packing. The ethanol vaporizes and flows to a condenser though the top of the flash vessel (a small air-bleed stream located near the bottom of the flash unit facilitates the flow of ethanol vapour to the condenser). Because of its high boiling point the solvent does not vaporize and collects at the bottom of the flash unit. It is returned to the solvent reservoir periodically by a peristaltic pump. Size 15 Viton® tubing is used in the pump head due to the higher temperatures encountered.

The process is controlled by CIM-PAC® software, as will be fully described in the next section. Information is gathered from five level switches and two flow switches which monitor the following:

Solvent Reservoir High Level,
Solvent Reservoir Low Level,
Solvent Surge High Level,
Solvent Surge Low Level,
Flash Unit High Level,
Solvent To Flash;Flow/No-Flow,
Solvent Return (to solvent reservoir);Flow/No-Flow.

In addition to these control-oriented sensors, CIM-PAC® monitors fermentor temperature and pH (which are controlled automatically by an MBR MCS 10 control cabinet), and flash temperature and pressure. The control algorithm employed is listed in Appendix C.

An operating manual for the PDU was devised and is available upon request. Although intended for PDU operation, it would be useful for larger-scale facilities as well.

4.2 EQUIPMENT UPGRADING

An Alfa-Laval LAPX-202 BGT-24F continuous centrifuge was installed to remove water and cells from the solvent, as well as to concentrate the yeast cells for cell recycle. This was a vast improvement over the staged settling column which was used previously and which usually resulted in a "dirty" solvent stream. The centrifuge can achieve a maximum force of 8200 G at 10,000 rpm. The parts in contact with solvent are either stainless steel, Viton® or C-flex® to prevent corrosion.

A 10 L stainless steel vacuum flash vessel was also installed to replace a similar glass unit. The new vessel was equipped with two 3-inch diameter sight glasses rated at 50 psig and an immersion heater for quick initial heat-up. The vessel was wrapped in fiberglass insulation to prevent heat loss. The solvent stream entered in the side of the vessel approximately two-thirds of the way from the bottom. A vapour withdrawal port was located at the top of the vessel while solvent was withdrawn from the bottom. The vessel also had an RTD temperature transmitter and a diaphragm/capacitance type pressure transmitter to monitor temperature and pressure of the flash vapour. Three ProMinent® Gamma 4-I diaphragm pumps were installed to withdraw solvent from the fermentor, to return cells to the fermentor, and to bleed cells to drain. The peristaltic pumps used previously had the problem of hoses sliding off their connections due to the lubricating properties of the solvent. The diaphragm pumps eliminated this problem because they are hard-plumbed into the system. Another advantage is that they are capable of fine flow control which is useful for maintaining a constant cell concentration in the fermentor.

A computer operated process control system was installed to provide continuous data-logging and control of the PDU. The control system used CIM-PAC© software, which is a commercial process control software package. CIM-PAC© is completely menu-driven and provides analog and digital control as well as real-time graphics, as shown in Figure 4-3. The continuous data acquisition feature, shown in Figure 4-4, has been extremely useful in identifying process upsets during the hours of unattended operation. The process control system obtained fluid flow information via field devices, specifically hi/low tank level switches and flow/no-flow switches. Flow control was achieved by digital control of all pumps. Special safeguards were built into the control algorithm, such as a loop to turn on the electrical heating tapes only if both the heating section pump was on and the corresponding flow switch indicated flow. If CIM-PAC© determined the system was out of control then an emergency shutdown was initiated in which all pumps, the heating tapes, and the centrifuge were stopped and the fermentor allowed to run batch.

4.3 MODIFICATIONS AND TESTING

Over the course of several runs, numerous design improvements were made to the Process Demonstration Unit. These changes were made primarily to the major pieces of equipment; namely, the fermentor, the centrifuge, and the vacuum flash unit.

4.3.1 Fermentor

The fermentor is both the reaction vessel, with ethanol produced by fermentation, and the extraction vessel, with solvent rising though the broth to remove the ethanol. To ensure a high degree of mass transfer between the solvent and aqueous phases, solvent recycle was initially employed. That is, the solvent layer which had formed on top of the fermentation broth was recycled back though the broth at a rate of 10 - 20 L/h. Although this ensured a high ethanol distribution coefficient (approximately 0.27) it also caused an excessive amount of fermentation broth

FIGURE 4-3

PROCESS FLOW DIAGRAM GENERATED BY CIM-PAC©

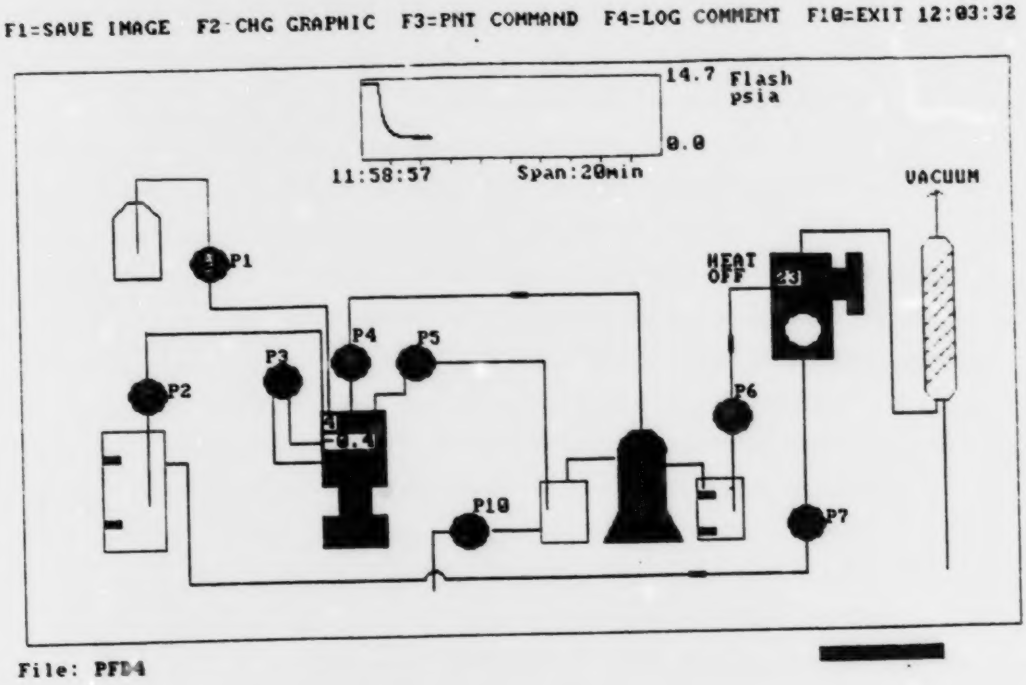
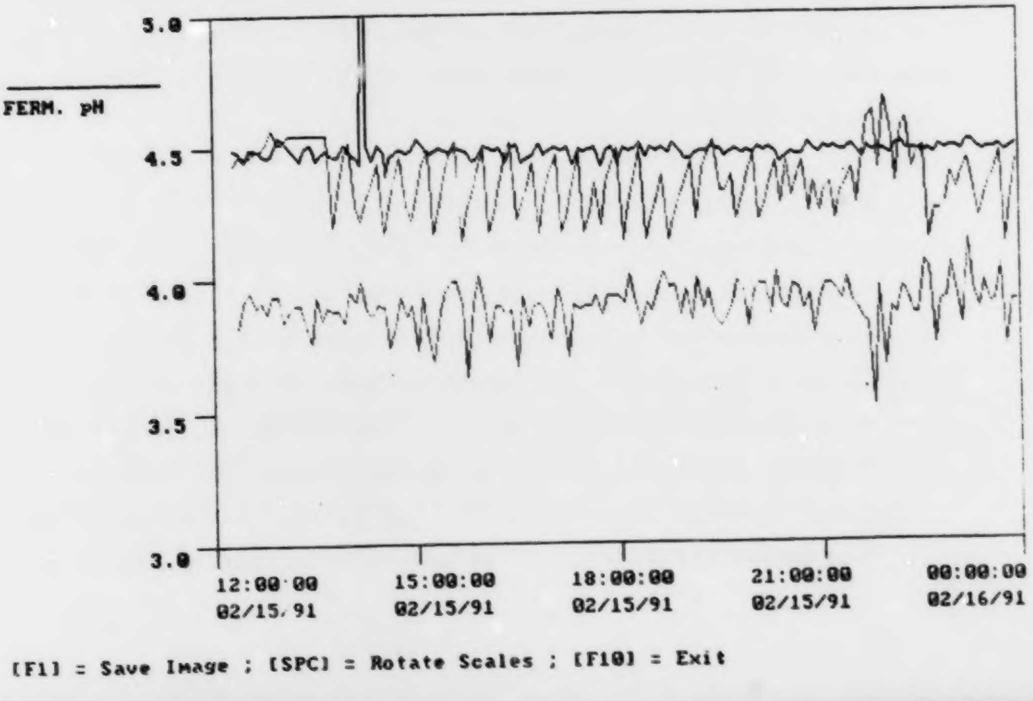

F1=SAVE IMAGE F2-CHG GRAPHIC F3=PNT COMMAND F4=LOG COMMENT F10=EXIT 12:03:32
14.7 Flash
psia
0.0
11:58:57
Span:20min
VACUUM
P1
HEAT
OFF
P4
P5
P3
P2
P6
P10
P7
File: PFD4

FIGURE 4-4

EXAMPLE OF DATA AQUISITION BY CIM-PAC©



to become entrained in the solvent stream leaving the fermentor, which in turn made level control difficult. Later runs without recycle resulted in comparable distribution coefficients (0.22 - 0.26); thus, solvent recycle was subsequently eliminated, and thus the process was simplified.

Air was initially supplied to the cell culture though a ring sparger located at the bottom of the fermentor. This sparger also supplied the solvent, which resulted in finely dispersed solvent droplets thoughout the aqueous broth. Although this helped to ensure good mass transfer, it also caused tubing to collapse in the solvent-to-fermentor pump. The air was later supplied by a separate line; no change in the ethanol distribution coefficient was observed.

4.3.2 Centrifuge

Initial tests using solvent/medium/yeast mixtures revealed that adequate separation of the medium and cells from the solvent could be obtained with a speed of as low as 5000 rpm. Centrifugation did not harm the yeast, as yeast viability remained constant even after repeated passages though the unit. Nuclear magnetic resonance spectroscopy alluded that only a trace of water was present in the solvent after centrifugation. Other tests provided different conclusions, however. Computer simulations with UNIFAC predicted that at equilibrium the solvent phase contains about 70% ethanol and 30% water (w/w); thus, the maximum concentration of ethanol in the condensate should be about 70% (wt). Some experiments with ethanol/solvent/water mixtures seem to support this.

Actual centrifuge operation within the PDU did not provide the expected results, however. Although the condensate did reach a maximum of 67% (wt) ethanol during one run, the concentration was on average 50% (wt). As well, water and occasionally cells were observed in the centrifuged solvent. Thus, an extensive series of centrifuge tests were conducted to determine why adequate separation was not achieved during PDU operation.

One test revealed that the centrifuge feed was being diluted with the process water which was used to hydraulically close the bowl. A feed of 80 g/L ethanol in water, for example, could be diluted to 54 g/L after a single pass though the centrifuge. Traces of ethanol in the process water drain line were also detected, indicating a mixing of the feed and process water. Misting of the process water was thought to be one of the causes of the problem. By operating the centrifuge without the collection trays in place, it was shown that some misting did occur. Changing the viton seals and o-rings and sealing the bowl-seat with vacuum grease provided some improvement.

Another test revealed that not all of the water was from the centrifuge. A solution of solvent (containing no water) and ethanol was prepared and flashed in the vacuum flash unit. The resulting condensate contained 17% (wt) water, likely caused by moisture-laden air leaking into the system. It was also found that the ethanol concentration in the condensate could be increased by decreasing the vacuum in the flash unit. By decreasing the vacuum from -87 kPa to -50 kPa, the ethanol concentration in the condensate increased from 50% (wt) to 78 % (wt). However, the lower vacuum also resulted in only about 25% of the ethanol being removed from the solvent.

4.3.3 Vacuum Flash Unit

The vacuum flash unit had undergone several changes to reach a final working design. Initially, hot solvent entered the unit about two-thirds from the vessel bottom via 3/8 inch stainless steel tubing. About four inches of the tubing, with several small holes drilled along its length, protruded inside the unit. Drops of solvent flowed out of these holes and dropped by gravity to the bottom of the unit. Testing showed that much of the solvent flowed out of only one hole however, resulting in poor ethanol removal.

To increase the exposed surface area and hence ethanol removal, steel wool was placed over the tubing. The hot solvent was allowed to trickle over the wool as it flowed to the bottom of the flash unit. After one run, however, it was observed that the packing had corroded substantially.

The steel wool packing was subsequently replaced by a spray nozzle. At room temperature the solvent exited the nozzle as a stream, which progressed to a spray as the temperature increased (and thus the viscosity decreased). With the nozzle in place over 85% of the ethanol was removed from the solvent when the flash was operated in the neighbourhood of 121 °C and -87 kPa vacuum. After 330 hours of running, however, the spray nozzle had become blocked with a black, charred substance, believed to be cells in the solvent from improper centrifuge operation. The same residue had also coated the heating section tubes. Thus the spray nozzle was impractical as it was prone to clogging.

The spray nozzle was replaced by a stainless steel basket which contained glass beads. The hot solvent flowed over the beads, creating a large surface area. This was the design finally employed in PDU operation. Like the spray nozzle, the glass bead packing also provided at least 85% ethanol removal at flash conditions.

A series of tests was undertaken to characterize flash efficiency in terms of operating pressure and temperature. Although not essential for PDU operation, this information would be useful for pilot and industrial scale facilities. The results from these tests are presented in Appendix D.

4.4 MEDIUM FORMULATION

A synthetic medium with glucose as the carbon source was used for both conventional and Extractive Fermentation operation. Elemental requirements were supplied by various salts. Yeast extract (type Y-4000, Sigma Chemical Co., St. Louis, MO) was also supplied as a source of organic nutrients.

Originally the medium for Extractive Fermentation was formulated such that the salts and yeast extract were increased according to the glucose concentration. For example, Table 4-1 compares the medium formulation used for conventional fermentation (194 g/L glucose) to that originally used for Extractive Fermentation (500 g/L glucose). As shown, all nutrients were increased by a factor of 500/194 for Extractive Fermentation. This formulation, however, contributed to high levels of unused salts in the fermentor. For example, Figure 4-5 shows the approximate fermentor concentration of salts and yeast extract over PDU run # N647GSC, during which the medium formulations presented in Table 4-1 were used. Also indicated are the concentrations of ammonium sulfate required to cause a 20% and a 50% reduction in cell yield for some strains of *S. cerevisiae* (Maiorella, 1984b). Cell yields during the Extractive Fermentation portions of this run were significantly less than expected, likely due to the inhibitory osmotic effect of accumulated high salt concentrations.

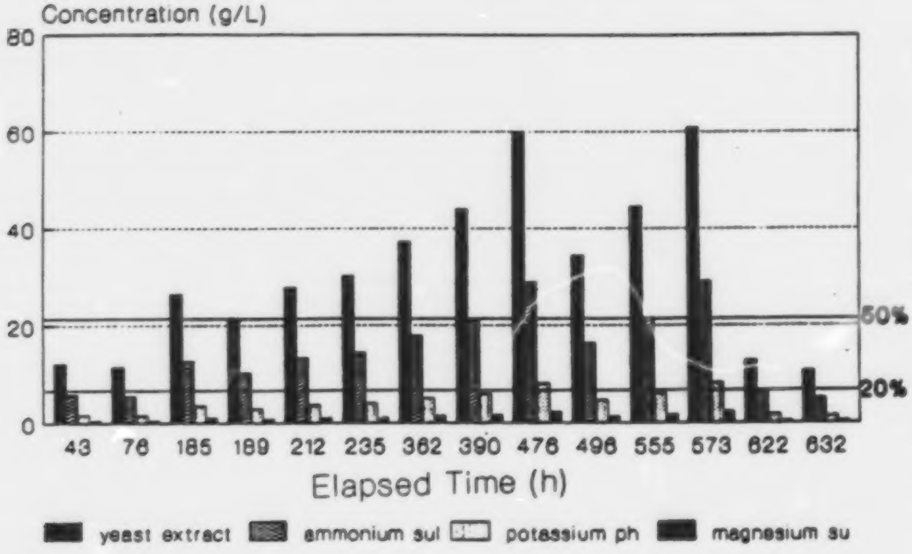
TABLE 4-1

MEDIUM FORMULATION FOR CONVENTIONAL FERMENTATION COMPARED TO "ORIGINAL" MEDIUM FOR EXTRACTIVE FERMENTATION

Component	Conventional Concentration (g/L)	Extractive Concentration (g/L)
Glucose	194	500
$(NH_4)_2SO_4$	6.71	17.30
KH_2PO_4	1.98	5.10
$MgSO_4 \cdot 7H_2O$	0.71	1.83
Yeast Extract	12.93	33.33
$ZnSO_4 \cdot 7H_2O$	9.70 mg/L	25.00 mg/L
$CuSO_4 \cdot 7H_2O$	2.43 mg/L	6.26 mg/L
$CoCl_2 \cdot 6H_2O$	2.43 mg/L	6.26 mg/L
$MnSO_4 \cdot 4H_2O$	0.49 mg/L	1.26 mg/L

FIGURE 4-5

Approximate Concentration of Salts and Yeast Extract During Run # N647GSC



Concentration of ammonium sulfate required to produc 50% reduction in cell yield

Concentration of ammonium sulfate required to produc 20% reduction in cell yield

Assuming equal salt utilization

High levels of salts can inhibit cell and ethanol production. Levels of ammonium sulfate over 6 g/L in the feed can cause a 20% reduction in cell yield, and an 80% reduction can occur when levels exceed 29 g/L; elevated levels of sodium chloride and other salts in the fermenting medium can also increase the production of glycerol as a fermentation by-product, at the expense of ethanol production (Maiorella, *et al.*, 1984b). Although sodium chloride was not directly added to the medium, it could have been a major component of the yeast extract. Some types of yeast extract can contain as much as 37% (wt) sodium chloride (Peppler, 1979).

A new medium formulation was devised to minimize the feed component inhibition that was observed during run # N647GSC. With this new formulation, only the glucose concentration was increased for Extractive Fermentation, with all other components at the same concentration as for conventional fermentation. That is, the glucose concentration was increased to 500 g/L, while all other components were at the conventional concentration shown in Table 4-1. The result was a 30% increase in cell viability and improved substrate conversion.

4.5 ANALYTICAL METHODS

This section describes the analytical procedures for measuring ethanol, cell, and residual glucose concentrations, as well as cell viability and budding.

4.5.1 Ethanol Concentration

Aqueous ethanol concentration was measured with a Gow Mac gas chromatograph equipped with a series 750P flame ionization detector and connected to a Hewlett-Packard 3390 A Integrator. The unit used a 1.2 m x 3.2 mm column packed with Poropak Q (80-100 mesh). Injector, detector, and oven temperatures were 160 °C, 190 °C, and 130 °C, respectively. The flowrates of hydrogen, air, and nitrogen (the carrier gas) were 20 mL/min, 240 mL/min, and 30 mL/min, respectively. Isobutanol was added to the aqueous samples (4.76 % v/v) as an internal standard. Samples were analyzed by injecting 3 μL of the sample into the column. The ratio of ethanol to isobutanol peak areas was recorded by the integrator. The corresponding ethanol concentration was read from a standard curve with ethanol concentrations in the range of 0 - 80 g/L. The ethanol concentration in the condensate was measured in a similar fashion with the exception that the sample was first diluted with distilled water to less than 80 g/L ethanol.

It was not possible to analyze solvent samples directly for ethanol because ADOL® 85 NF boils at about 300 °C while the maximum operating temperature of the gas chromatograph was 250 °C. Instead 5 mL of the solvent sample was equilibrated with 5 mL of distilled water with a Mixxor™ separatory system at room temperature. The 10 mL mixture was then centrifuged for 5 minutes at 3000 rpm in a Damon centrifuge to separate the aqueous and solvent phases. A 3 μL sample of the aqueous phase was then injected into the gas chromatograph column, with settings as already described. The resulting ethanol peak was compared to a standard curve prepared in the same manner; that is, by equilibrating distilled water with solvent which contained known concentrations of ethanol in the range of 0 - 15 g/L, at room temperature. Although actual experimental data are unavailable, the amount of ethanol which dissolved in the distilled water phase should have been relatively insensitive to small changes in room temperature. Computer simulations have shown that a temperature increase from 20 °C to 60 °C causes the mass-based ethanol distribution coefficient to decrease by only 2.5%.

4.5.2 Glucose Concentration

The concentrations of D-glucose in the aqueous phase were analyzed by the DNS method (Miller, 1959). Samples which had been centrifuged to remove cells were diluted to between 0 and 1 g/L; 1 mL of this was then added to 3 mL of DNS reagent and placed in a boiling water bath for 15 minutes. Glucose standards in the concentration range of 0 - 1 g/L were prepared in a similar fashion and run with each sample. After cooling to room temperature, the absorbance of the samples were measured with a Brinkman PC 800 Colourimeter at 650 nm and compared to the measured absorbance of the glucose standards.

4.5.3 Cell Concentration

Yeast cell concentration was measured by turbidometry. A standard curve was prepared by centrifuging 10 mL of a cell suspension for 10 minutes at 3000 rpm. The supernatant was discarded and the cell pellet resuspended in distilled water and centrifuged again for 10 minutes at 3000 rpm. Once more the supernatant was discarded and the cells resuspended in distilled water.

The suspension of "washed" cells was dried at 105 °C to constant weight and the cell concentration in the original cell suspension was thus measured. From the same cell culture, suspensions of known cell concentration in the range of 0 - 1 g/L were prepared with "washed" cells, as described above, and the absorbance at 650 nm was measured. The standard curve was formed from these measurements. Cell concentration in a sample was subsequently measured by "washing" the cells from a 10 mL sample, diluting that to between 0 and 1 g/L, and measuring the absorbance at 650 nm. The measured cell concentration was read from the standard curve. The same procedure was used for *Zymomonas mobilis* with the exception that the cells were centrifuged at 12000 rpm.

The live cell concentration was used in determining PDU operation. That is, the cell concentration in the fermentor was taken to be the measured cell concentration, as described above, multiplied by the cell viability.

4.5.4 Cell Viability and Budding

Both cell viability and cell budding were measured to determine the general health of the yeast cell culture. Cell viability was measured by a methylene blue staining procedure (Alltech Biotechnology Center, Nicholasville, Kentucky). Approximately 0.5 mL of fermentation broth was mixed with 10 mL of a modified Ringer's solution containing 0.25 g/L methylene blue. After 10 minutes the mixture was diluted to obtain 50 - 100 cells in the counting area of a Neubauer haemacytometer. Cells which were stained blue were counted as dead cells. Cell viability was taken as the number of unstained cells divided by the total number of cells.

Cell budding was measured at the same time as cell viability. Only live cells were counted. Any cell on which a distinct bud or buds had formed or any budded cell which had not completely separated from its parent cell was counted as a single budded cell. Cell budding was taken as the number of live budded cells divided by the total number of cells.

4.6 RESULTS

Since the initial commissioning of the PDU a total of six runs were performed with synthetic medium and yeast. Information from only one run is presented however, as the other runs consisted mostly of testing and fine-tuning. Highlights from some of the other runs can be found in Appendix E. Data from all runs are available upon request.

4.6.1 Fermentor Conditions

One operation of the Extractive Fermentation PDU began on June 3, 1990 and lasted for a total of 198 hours. The purpose of the run was to confirm the optimum operating conditions predicted by the PROCESS™ computer simulations.

The fermentor conditions observed over the course of the run are presented in Figure 4-6. The run was divided into four periods: an initial period of dynamic conventional operation, a period of steady state conventional operation, a transition period from conventional to Extractive Fermentation, and a final period of steady state Extractive Fermentation.

Initial Conventional Period

The purpose of the initial conventional operation was to bring the live cell concentration up from the initial concentration of 4 g/L (following 20.5 hours of batch fermentation) to the desired operating concentration of 65 - 75 g/L. During this period the cell concentration increased at a rate of approximately 0.84 g/L·h. At 51.5 hours approximately 33 g of cells in the centrifuge cell discharge were not returned to the fermentor to help prevent fermentation by-products from accumulating in the fermentor. The target cell concentration of 65 g/L was achieved at 69.0 hours.

Steady State Conventional Operation (69.0 - 96.0 hours)

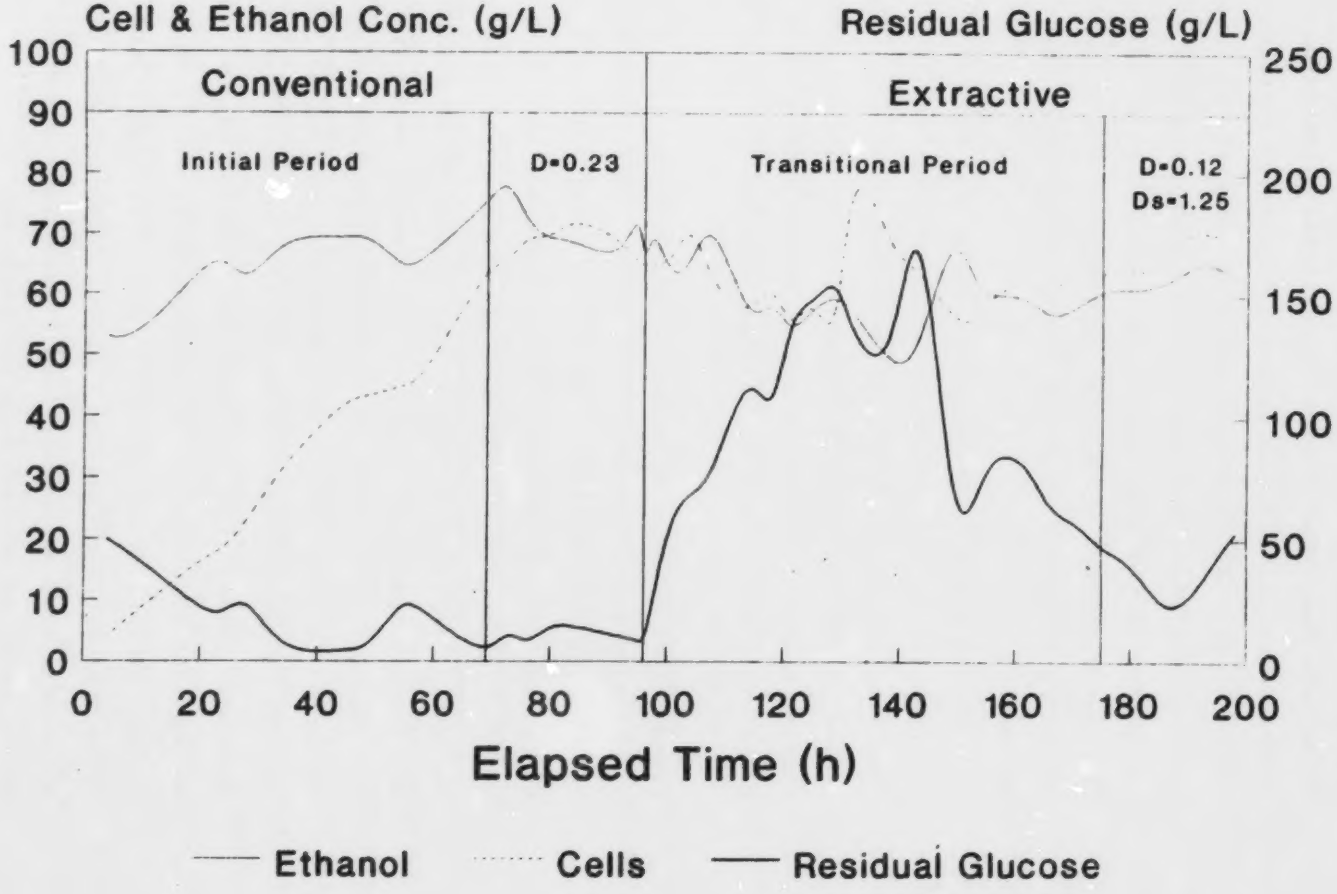
Steady state conditions were assumed after 3 reactor volumes of medium had passed though the fermentor if no major changes to the fermentor operating conditions were made during that time and if thee relatively consistent fermentor samples had been obtained. During the conventional steady state period the aqueous dilution rate was 0.23 h^{-1}, while the average feedstock concentration was 194 g/L (18% wt) glucose. The mean ethanol, cell, and residual glucose concentrations were 71 g/L, 68 g/L, and 10 g/L, respectively. The fermentor aqueous volume remained steady at 4.8 L. Roughly 25 g of cells were removed from the system at 77.0 hours and another 37 g were removed at 84.0 hours for a total of 62 g.

Transitional Period (96.0 - 175.0 hours)

This period marked the switch from conventional to Extractive Fermentation. At 96.0 hours the medium was switched from 194 g/L glucose to

FIGURE 4-6

FERMENTOR CONDITIONS
DURING RUN # J198GSC



493 g/L glucose. The aqueous dilution rate was reduced from 0.23 h^{-1} to 0.15 h^{-1}, while the solvent flow was started at a dilution rate of 1.25 h^{-1}; these were the optimum operating conditions predicted by the computer simulations. As shown in Figure 4-6, a rapid increase in residual glucose soon followed, although this steadied about midway though the period. There was also a gradual decrease in both ethanol and cell concentrations, which was caused at least in part by an accumulation of cells in the centrifuge. The large increase in cell concentration at 131.5 hours was caused by returning the cells which had become caked inside the centrifuge back to the fermentor.

At 143.0 hours the residual glucose had reached 209 g/L. The system was subsequently allowed to run in batch mode between 145.0 and 150.0 hours; in that time the residual glucose decreased to 52 g/L. The system was then restarted under Extractive Fermentation conditions with the aqueous dilution rate at 0.12 h^{-1} and the solvent dilution rate at 1.25 h^{-1}. The system ran undisturbed under these conditions from 149.0 - 198.0 hours.

Steady State Extractive Fermentation Operation (175.0 - 198.0 hours)

Steady state Extractive Fermentation operation was maintained during this period for 23 hours. The mean ethanol, cell, and residual glucose concentrations during steady state operation were 62 g/L, 67 g/L, and 38 g/L, respectively. The mean glucose concentration in the feedstock was 503 g/L. Since the fermentor cell concentration did not increase outside the desired operating range, no cells were removed during this period.

To summarize, the mean fermentor conditions observed during the run are presented in Table 4-2. For comparison, the values predicted by the PROCESS™ computer simulations are also shown. There is relatively good agreement between the predicted and observed values, considering the numerous assumptions involved in the computer model (which do not take into account changes in cell viability or the effect of high ethanol concentrations on biomass production) and the unknowns connected with Extractive Fermentation operation, such as optimal feed formulation, amount of aeration required, effect of pH on ethanol mass transfer, etc.

TABLE 4-2

STEADY STATE FERMENTOR CONDITIONS AND PREDICTED CONDITIONS

	Conventional		Extractive	
	(Predicted)	(Actual)	(Predicted)	(Actual)
Ethanol Conc. (g/L)	82	71	75	62
Cell Conc. (g/L)	75	68	75	67
Residual Glucose (g/L)	6	10	8	38
Feedstock (Glucose, g/L)	194	194	500	503
Aqueous Dilution Rate (h^{-1})	0.20	0.23	0.15	0.12
Solvent Dilution Rate (h^{-1})	-	-	1.25	1.25
Substrate Utilization (%)	97	95	98	92

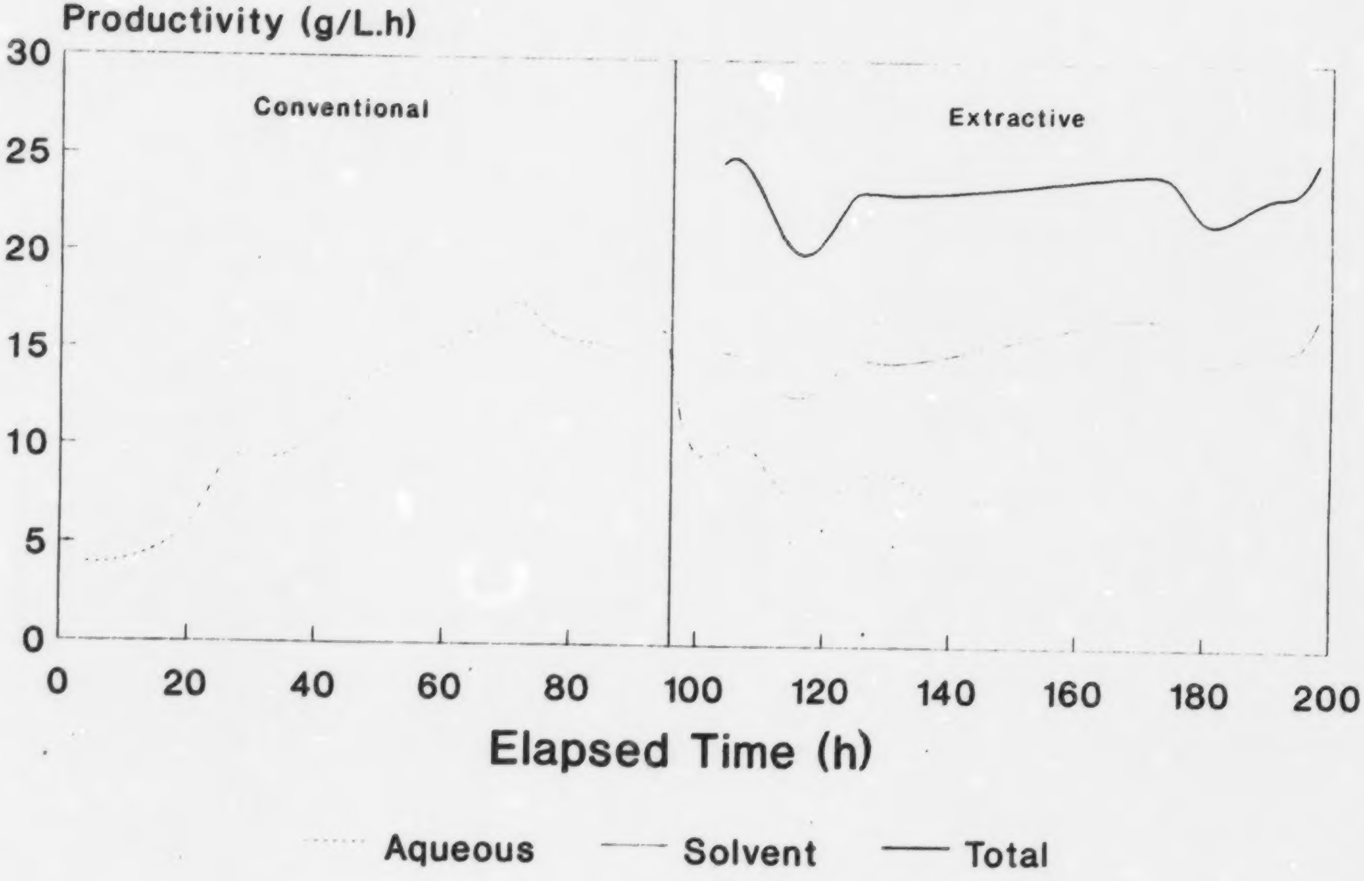
4.6.2 Ethanol Production

The ethanol productivities obtained over the duration of the run are presented in Figure 4-7. Total ethanol productivity refers to the ethanol production rate as determined by the amount of ethanol in both the solvent and aqueous streams, normalized with respect to fermentor volume.

During the conventional steady state period the mean ethanol productivity was 16.0 g/L·h. The ethanol yield was 0.39 g ethanol/g glucose. The total ethanol productivity increased to an average of 23.2 g/L·h during steady state Extractive Fermentation operation. The mean mass-based ethanol distribution coefficient was 0.23, which was achieved with a single pass of solvent though the fermentor. Under the flash conditions of 126 °C and approximately 21 kPa absolute pressure, 86% of the ethanol was removed from the solvent on average. Between 176.0 and 198.0 hours a total of 1133 g of ethanol was collected in the condenser. Based on the amount removed from the solvent during this time, 29% of the ethanol was lost though the condenser, which was cooled by water at 12 °C. Also in this time a total of 2395 g of ethanol was removed from the system (799 g in the aqueous bleed stream and 1596 g in the solvent stream), while 5998 g of glucose were used, for an ethanol yield of 0.40 g ethanol/g glucose.

FIGURE 4-7

ETHANOL PRODUCTIVITY
DURING RUN # J198GSC



The average ethanol concentration in the condensate between 176.0 and 198.0 hours was 309 g/L (34% wt). Improper centrifuge operation was the cause of the low concentration as cells and fermentation broth were observed in the solvent in this time period. Over the entire Extractive Fermentation period, including the Transient Period, the mean ethanol concentration in the condensate was 395 g/L (44% wt). As previously mentioned, higher concentrations (up to 67% wt) have been achieved; the factors influencing the final ethanol concentration have not yet been fully investigated.

4.6.3 Cell Production

Although the cell concentration quickly increased during the initial conventional period, cell production seemed to slow as the ethanol concentration increased. Cells were only removed from the system twice during the conventional steady state period, with the cell yield at 0.0362 g cells/g glucose, which was lower than the expected value of 0.0572 obtained in other experiments (Kollerup, 1986). Cell production appeared to be negligible during the Extractive Fermentation steady state period and no cells were removed from the system; this could, however, have been caused in part by an accumulation of cells in the centrifuge, as already mentioned. It has been observed that if too many cells remain in the centrifuge, some overflow into the centrifuge water drain line.

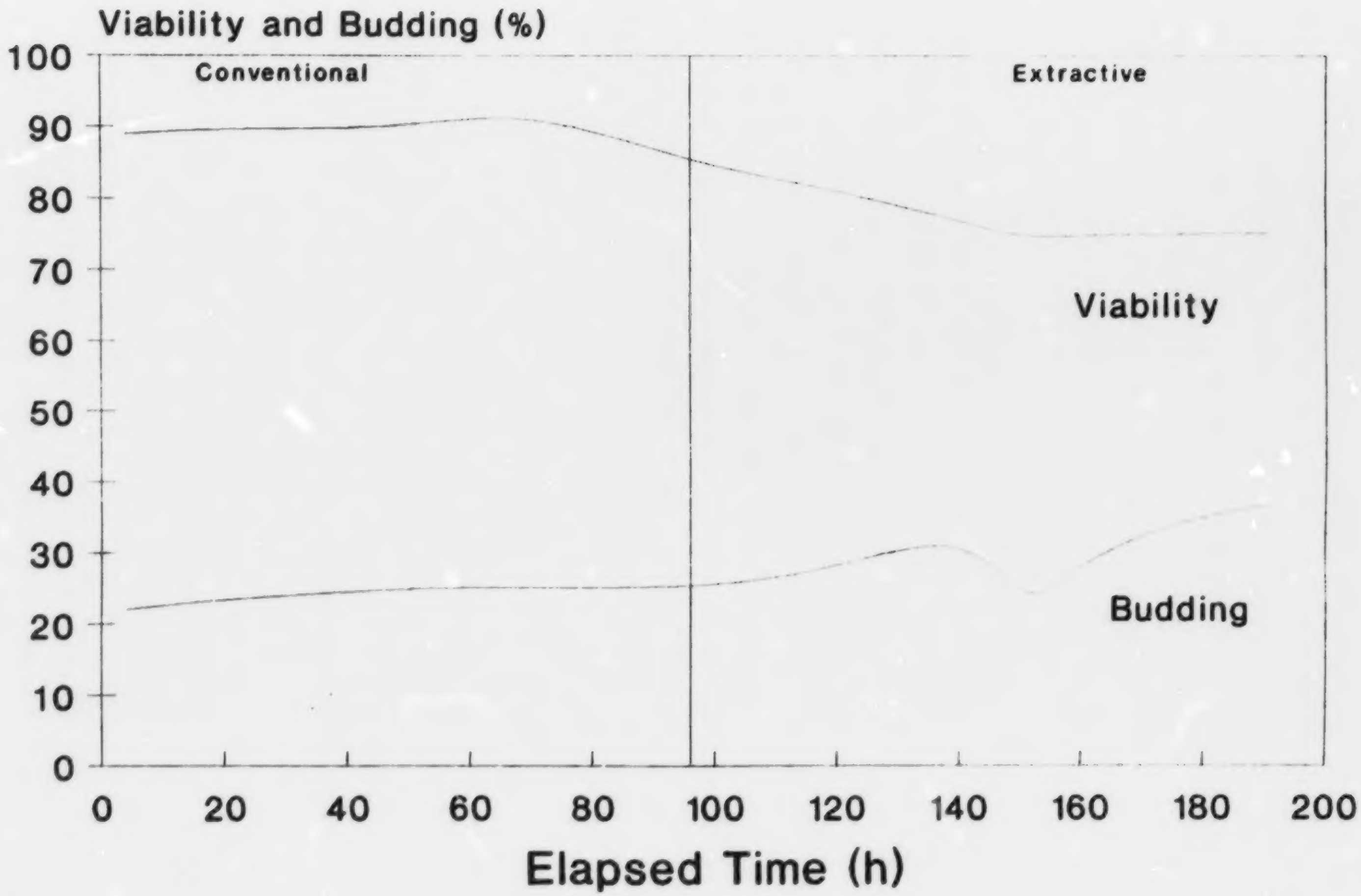
4.6.4 Viability and Budding

Figure 4-8 presents the measured cell viability and budding over the course of the run. Both remained fairly constant during the conventional period, although viability increased slightly to 93% at 69.0 hours. Average values for the conventional steady state period were 89% viable cells and 24% budded cells.

There was a gradual decrease in cell viability during Extractive Fermentation operation, until about 153.0 hours, at which point it stabilized at about 75%. At the same time, cell budding gradually increased, except for the decrease observed after the fermentor had run batch between 145.0 and 150.0 hours. Average viability and budding during steady state operation were 75% and 37%, respectively. The phenomena of decreased cell viability and increased cell budding during Extractive Fermentation have been observed in other runs.

FIGURE 4-8

CELL VIABILITY AND BUDDING
During Run # J198GSC



4.6.5 Centrifuge Operation

The operating conditions for the centrifuge had to be changed periodically to accommodate changing conditions in the fermentor. Over the initial conventional period the centrifuge was operated at 5500 rpm with a discharge time of 20 minutes. At 62.5 hours however, cells were observed in the centrifuge solvent tray, and the discharge time was decreased to 15 minutes. The measured cell concentration at this time was 50 g/L.

At 129.5 hours it was observed that the fermentor cell concentration was decreasing. It was discovered that cells had accumulated in the centrifuge, to the point of almost completely filling the cell tray. The discharge time was again decreased to 10 minutes and later to 7.5 minutes to prevent the concentrated cell stream from becoming too viscous and to help flush out the centrifuge.

As previously mentioned, cells and fermentation broth were observed in the solvent during steady state Extractive Fermentation operation. This may have been caused by the bowl being too full with cells.

4.6.6 Solvent Losses

The concentration of solvent in the ethanol condensate during the run was measured by high temperature gas chromatography to be 13 - 18 ppm. Results from other runs have shown that substantially higher solvent levels in the ethanol condensate sometimes do occur, but this is attributed to misting and/or entrainment, which can be resolved by a slightly modified flash design and some fine-tuning of operating conditions. The concentration of residual solvent in the fermentor aqueous broth (after centrifugation) was 30 - 62 ppm.

4.7 DISCUSSION OF RESULTS

The fermentor conditions obtained during PDU operation were comparable to those predicted by computer simulation, and thus verified the validity of those simulations. The results also demonstrated that stable operation of the PDU under Extractive Fermentation conditions with cell recycle could be maintained without a significant loss in cell viability. It was also shown that ethanol production by Extractive Fermentation with cell recycle under optimal conditions is superior to conventional fermentation with cell recycle. Extractive Fermentation resulted in an increase of approximately

50% in ethanol productivity over conventional fermentation, and allowed greater than 90% conversion of a concentrated (41.5% wt glucose) feedstock.

Cell production was lower than expected during both conventional and Extractive Fermentation; however, this could be explained by the high ethanol concentration. The cellular growth rate of some strains of *Saccharomyces* can be inhibited by more than 50% at ethanol concentrations in the neighbourhood of 60 g/L (Bazua and Wilke, 1977). This may prove to be yet another advantage of Extractive Fermentation in that the ethanol concentration, and thus presumably cell production, can be easily controlled by adjusting the solvent dilution rate (unlike conventional fermentation, in which the aqueous flowrate must be adjusted). This would allow facilities to easily increase the amount of biomass produced if desired, while still maintaining complete substrate conversion.

Some of the discrepancy between predicted and actual fermentor conditions may be explained by inaccurate measurement of the aqueous volume in the fermentor. As previously mentioned, the aqueous volume tended to decrease during Extractive Fermentation. To prevent this, a small flow of "spent" fermentation fluid was returned to the fermentor to maintain a constant aqueous level (approximately 4.8 L). It was, however, often difficult to determine the actual aqueous volume in the fermentor since the aqueous/solvent interface was frequently diffuse, and on occasion the entire fermentor contents appeared homogeneous. Periodically, the flows to the fermentor were stopped and all agitation halted to allow the two phases to separate. Though this procedure, aqueous volumes as low as 4.4 L were recorded, and may have been lower; thus, the aqueous and solvent dilution rates during Extractive Fermentation may have been slightly higher than those reported.

The loss of solvent in the aqueous stream appears to be slight, as the solvent is virtually insoluble in water. Larger losses may appear though the flash/condensor system, but could possibly be minimized with further fine-tuning.

5.0 NATURAL SUBSTRATES

Although the Extractive Fermentation PDU performed as expected according to the computer simulations, the medium used was not identical to that which would be used on a commercial scale. For industrial scale operation, natural substrates, specifically molasses and hydrolyzed grain products, are the substrates of choice (Maiorella, 1985; Parsons, 1988). Since one of the advantages of Extractive Fermentation is the ability to ferment concentrated (>40 % wt fermentables) feedstocks, these materials with their high sugar content (35 - 50 % wt) would be ideally suited for such a process. To observe the performance of natural feedstocks in an Extractive Fermentation system the PDU was operated with both molasses and hydrolyzate. These were preliminary tests aimed at, in the first instance, showing the feasibility of such operation and identifying problem areas requiring further research and development.

5.1 OPERATION WITH BLACKSTRAP MOLASSES

Blackstrap molasses is a by-product of sugar manufacturing. It contains a high concentration of fermentable sugars (typically >500 g/L) and other nutrients. It also contains several substances, mainly calcium salts, which inhibit fermentation (Maiorella, 1985).

Before PDU operation with molasses began, several bench-scale fermentations were undertaken to obtain information on operating conditions. The tests used molasses diluted with hot tap water to between 180 and 220 g/L total reducing sugars. The tests revealed that the molasses obtained from a local supplier (Topnotch Feeds Ltd., Kingston, Ontario) was more easily fermented than that from another source (JonTon Alcohol, Edinburg, Texas). It was also found that adding trace nutrients to the molasses did not significantly improve fermentation. Treating the molasses with K_2HPO_4 to remove calcium salts, however, did increase ethanol concentrations an average of 11% and decreased residual sugar concentrations an average of 22%. Another finding was that strains of yeast differ in their ability to ferment molasses. The strain *Saccharomyces cerevisiae* YT washed out at a dilution rate of 0.09 h^{-1} when using the "JonTon" molasses, while another strain supplied by Alltech (Alltech Biotechnology Center, Nicholasville, Kentucky) did not. Lastly, the test revealed that conditions suitable for Extractive Fermentation (i.e., aqueous ethanol >50 g/L) could be obtained either by maintaining the cell concentration around 70 g/L or by alternating continuous operation with brief periods of batch fermentation.

After the bench-scale fermentations, molasses was tested in the PDU. The molasses used was that obtained from Topnotch Feeds (Kingston). The molasses was diluted to the desired concentration with hot tap water. No other nutrients were added.

The run consisted of four main time periods. Initially there was a conventional period to determine adequate operating conditions. Following this was a period of Extractive Fermentation during which the only change to the fermentor was the addition of solvent. Next was a period of Extractive Fermentation using a "low concentration" molasses mixture (approximately 287 g/L total reducing sugars). The last period was also one of Extractive Fermentation, using a more concentrated (approximately 382 g/L total reducing sugars) molasses mixture.

All reported cell concentrations are measured cell concentrations unless otherwise stated. Live cell concentrations could not be employed because the cell viability measurements did not produce clear results. All analytics were identical to those performed with synthetic medium with the exception of residual sugars. Residual sugar concentration was measured by first inverting 1 mL of the diluted sample with 20 μL invertase for 10 minutes and then reacting the sample with DNS reagent as described by Miller (1959).

5.1.1 Fermentor Conditions

Conventional Period

Before conventional operation began there was a brief period of batch fermentation. The medium for this stage was composed of one part molasses diluted with thee parts water up to a volume of 5 L. The medium was sterilized in the fermentor at 121 °C for 20 minutes. After cooling, the fermentor was inoculated with "Alltech" yeast which had been grown in a 500 mL dilute molasses culture. The fermentation was batch for approximately 24 hours. One hour before conventional operation began, 450 g of dry "Alltech" yeast were added to the fermentor to increase the cell concentration to a reasonable level.

The fermentor was operated under conventional conditions for 27 hours at an aqueous dilution rate of 0.10 h^{-1}. The medium was prepared by mixing molasses and water in a 1:3 ratio, which yielded 157 g/L total reducing sugars (TRS). The medium was not sterilized; penicillin was added (16 mg/L) to prevent contamination. Since the addition of sulfuric acid to molasses results in the formation of precipitates, the fermentor pH was controlled by adjusting the pH of the incoming medium to about 4.0. Medium was withdrawn about 4 - 8 cm above the bottom of the medium carboy. In this manner, excess solids were prevented from entering or forming in the fermentor. A slow flow of concentrated cells was continuously recycled from the centrifuge cell discharge back to the fermentor to maintain a constant cell concentration.

The fermentor conditions are shown in Figure 5-1. Mean values over the entire period were 49 g/L (ethanol), 63 g/L (cells), and 44 g/L (residual sugars). The large initial increase in cell concentration was caused by the addition of more dried yeast at 2.0 hours.

Extractive Fermentation: 157 g/L TRS

At 27.0 hours Extractive Fermentation was begun with a solvent dilution rate of 1.25 h^{-1}. No other changes were made to the system. As shown in Figure 5-1 there was a gradual decrease in both ethanol and residual sugars to as low as 23 g/L and 18 g/L, respectively. Cell concentration remained relatively constant at an average of 71 g/L.

Extractive Fermentation: 287 g/L TRS

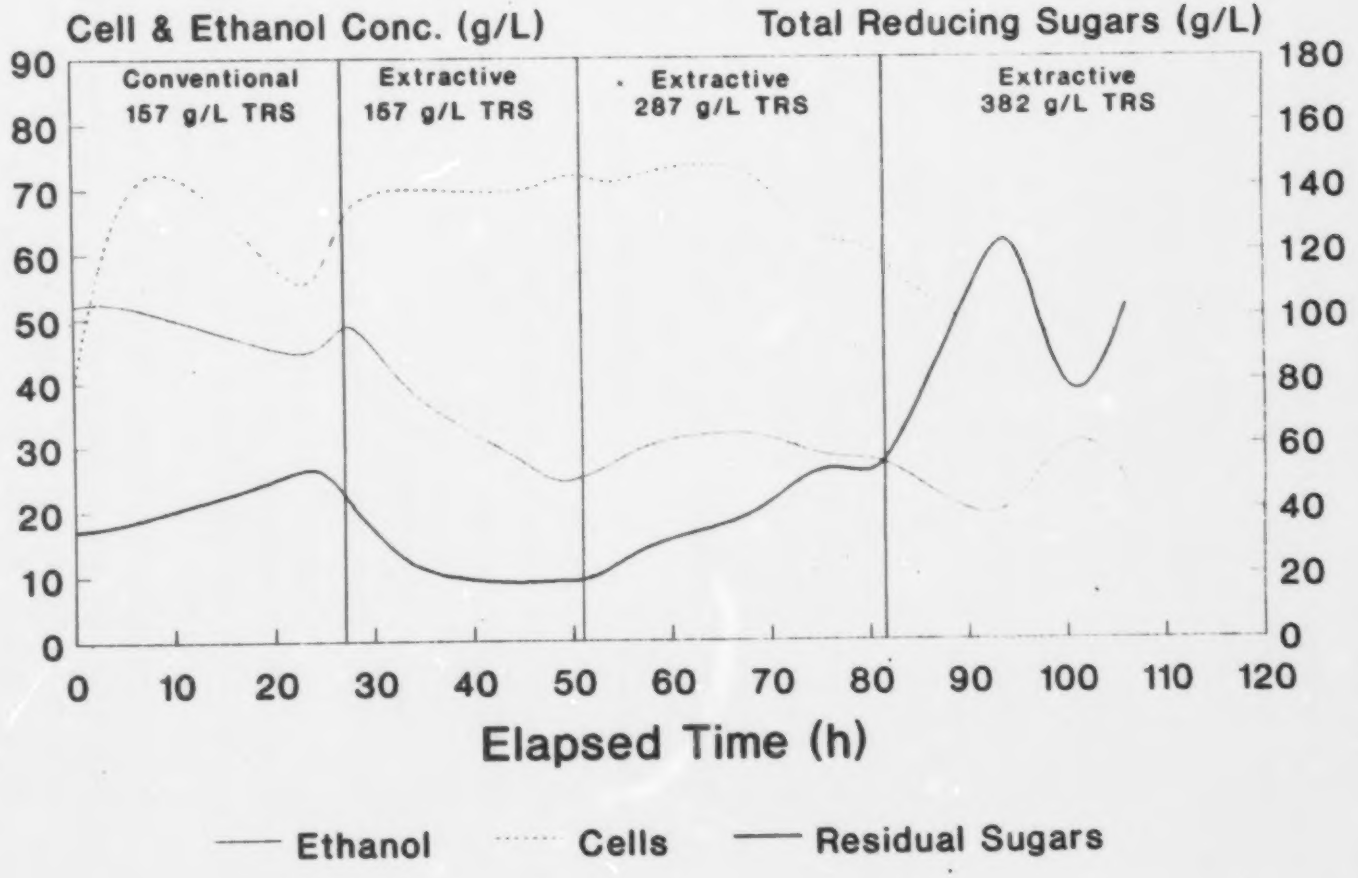
At 51.0 hours the 157 g/L TRS medium was substituted with a more concentrated medium. The medium was formulated by diluting molasses and water in a 1:1 ratio, resulting in 287 g/L TRS on average. As shown in Figure 5-1 there was a gradual increase in residual sugars over most of the period, although near the end it steadied at about 52 g/L. Ethanol concentration was relatively constant and had a mean value of 30 g/L. Although initially constant, the cell concentration decreased slightly after 68.0 hours. Mean cell concentration over the interval was 67 g/L.

Extractive Fermentation: 382 g/L TRS

At 81.5 hours the fermentor was again switched to a more highly concentrated feed. The new feedstock had a total sugar concentration of 382 g/L and was formulated by diluting molasses with water in a 1:0.4 ratio. Once again, the new medium resulted in an increased level of residual sugars as shown in Figure 5-1. The large increase at 90.0 hours was due to a process upset. The fermentor had filled with solvent overnight and approximately 80% of the fermentor aqueous volume had been lost. Some cells and aqueous fermentation fluid were returned to the fermentor, which was allowed to run batch between 98.0 and 100.0 hours. The cell concentration

FIGURE 5-1

FERMENTOR CONDITIONS
DURING MOLASSES RUN



Substrate: Untreated TOPNOTCH molasses

could not be returned to its former level however, and the system was shut down.

It is important to note that the residual sugar data generated during this interval (and perhaps during the 287 g/L Extractive Fermentation period) may be inflated due to the colour effect of the molasses on optical absorbance. A sample of spent fermentation fluid taken during this period and diluted in the same manner as samples prepared for sugar analysis yielded a "sugar concentration" of 32 g/L even when no DNS reagent had been added.

5.1.2 Ethanol Production

Ethanol production during thee of the four intervals is summarized in Table 5-1. There appears to be a trend, with ethanol yield decreasing as the molasses concentration in the feed increases. If this is an actual trend, it may be caused by salts present in the molasses which cause the cells to produce glycerol instead of ethanol. It has been shown that glycerol production increases at the expense of ethanol in hypertonic media (Maiorella, *et al.*, 1984b).

TABLE 5-1

ETHANOL PRODUCTION AND ETHANOL YIELDS

PERIOD	ETHANOL PRODUCED (g)	ETHANOL YIELD (g EtOH/g TFS*)	MEAN ETHANOL PRODUCTIVITY (g/L·h)
Conventional	635	0.35	4.9
Extractive (287 g/L)	1232	0.32	8.1
Extractive (382 g/L)	553	0.28	6.8

*Total fermented sugars

The average ethanol concentration in the solvent over the Extractive periods was 6.8 g/L, while the mean ethanol distribution coefficient was 0.24 on a mass basis. Based on the ethanol removed from the solvent in the flash unit, approximately 17% of the ethanol was not recovered.

5.1.3 Discussion Of Results

The PDU run with molasses demonstrated that Extractive Fermentation with molasses is possible, and that approximately 80% conversion of a medium strength (287 g/L fermentables) molasses solution could be achieved. Fermentation of a more concentrated (382 g/L fermentables) molasses solution did not provide good results.

A high concentration of salts in the molasses medium could have inhibited fermentation. As already mentioned, blackstrap molasses typically contains a high concentration of calcium salts, along with other products. Although these compounds are not a cause for concern in simple batch fermentation, they can be problematic in systems where yeast or stillage are recycled (Murphy, et al., 1982; Maiorella, 1985). One example of this can be seen in the Biostil® process, in which a stillage bleed stream is used to prevent the accumulation of non-fermentable components, while the remainder of the stillage is recycled back to the fermentor. If concentrated cane syrup is used as a substrate, the bleed stream can be reduced by a factor of 20; however, if blackstrap molasses is used, the bleed can only be reduced by a factor of 3 because of the high concentration of non-fermentable substances (Goggin and Thorsson, 1982). Systems which employ selective ethanol removal, such as Extractive Fermentation, are susceptible to inhibition by non-fermentable feed components because they use concentrated feeds and/or have small bleed-to-feed ratios. With the ethanol removed, the limiting influence on fermentation becomes the concentration of these components in the fermentor.

There are several solutions to the problem. One is to use a type of molasses that is low in non-fermentable compounds. There is a large variation in the quality of molasses available, depending on its point of origin (Van Ginneken, 1976). As already mentioned, the molasses supplied by Topnotch Feeds Ltd. was more readily fermented than that supplied by JonTon Alcohol Inc.

Another solution is to treat the molasses to remove the non-fermentable components. Two such methods are commonly used (Arroyo, 1949; Reich, 1942). Although both methods remove inhibitory compounds, they can also remove naturally occurring nutrients which may have to be supplemented before fermentation.

A third solution is to use a microorganism that is tolerant to the unwanted components. Osmotolerant yeasts are commonly used in industry to ferment "dirty" raw materials, such as blackstrap molasses, because they allow a large portion of the stillage to be recycled (Maiorella, 1985). As well, strains of *Zymomonas mobilis* are able to ferment blackstrap molasses without nutrient addition or treatment to remove salts (Doelle and Doelle, 1990). Some mixed cultures have also been shown to be superior in fermenting molasses compared to single strain fermentations (Layokun, 1984).

5.2 OPERATION WITH GRAIN HYDROLYZATE

Feedstocks prepared from hydrolyzed grain are another possible raw material for Extractive Fermentation. These materials are widely used in the fermentation industry, as more than 80% of the current ethanol production in North America is associated with corn wet milling (Parsons, 1988). The applicability to Extractive Fermentation was examined by using the material as a feedstock for a PDU run.

The hydrolyzate was donated by CASCO Inc. (Cardinal, Ontario) and was filtered before delivery to remove solids. Typically, this particular hydrolyzate contains 35% fermentables (Hobbs, 1990). A strain of *Saccharomyces cerevisiae* (Alltech Biotechnology Center, Nicholasville, Kentucky) was the fermenting organism. The fermentor was allowed to run batch for 21 hours after inoculating with a pure liquid culture. Approximately 1.5 hours before conventional operation began, 300 g of spray-dried Alltech yeast were added to the fermentor to increase the cell concentration to the desired level of 50 - 70 g/L.

The run consisted of five distinct periods beginning with a period of conventional operation to ensure the cells were active and viable. Next were thee periods of Extractive Fermentation at different dilution rates. The final period also consisted of Extractive Fermentation, but with no salts added to the medium.

5.2.1 Medium Formulation

For conventional fermentation the hydrolyzate was diluted with hot tap water to about 175 g/L glucose. The hydrolyzate was not diluted during Extractive Fermentation. Nutrients were added to the medium in the proportions shown in Table 5-2 because initial batch tests had provided poor results when hydrolyzate alone was fermented. The medium was not supplemented with nutrients during the final Extractive period, however, to determine if partial conversion of the substrate was still possible.

TABLE 5-2

Nutrients Added to Hydrolyzate

Nutrient	Amount Added
$(NH_4)_2SO_4$	6.73 g/L
KH_2PO_4	1.98 g/L
$MgSO_4 \cdot 7H_2O$	0.71 g/L
Yeast Extract	13.00 g/L
Trace Element Solution*	1.30 mL/L

*Consisted of 7.5 g/L $ZnSO_4 \cdot 7H_2O$, 1.88 g/L $CuSO_4 \cdot 7H_2O$, 1.88 g/L $CoCl_2 \cdot 6H_2O$, and 0.38 g/L $MnSO_4 \cdot 4H_2O$.

The medium used during the conventional period was pH adjusted with sulfuric acid to 4.0 and sterilized for 20 minutes at 121 °C. The medium was neither sterilized nor pH adjusted during Extractive Fermentation. To control contamination, penicillin was added to all media at a concentration of 16 mg/L. This was a precautionary measure since the concentration of fermentables, and thus the osmotic pressure, in the hydrolyzate was not as high as with the synthetic medium.

5.2.2 Fermentor Conditions

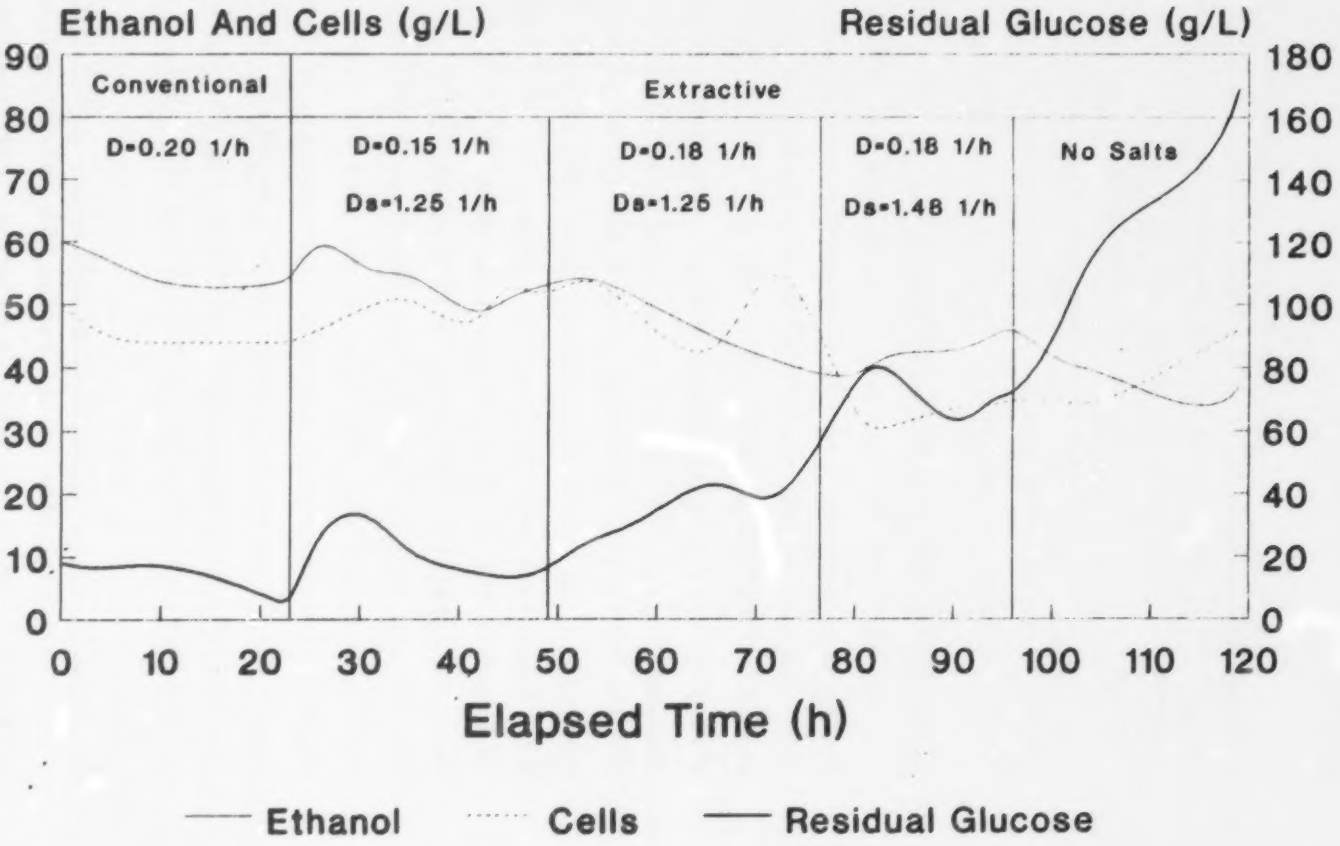
Conventional Period (0.0 - 23.0 hrs)

The purpose of this period was to ensure the cell culture was healthy and active and that conditions were relatively stable before Extractive Fermentation was begun. The aqueous dilution rate during this period was 0.20 h^{-1}, while the medium was at 175 g/L glucose. As shown in Figure 5-2 this period was typified by relatively stable ethanol, cell, and residual glucose concentrations, which at steady state were 54 g/L, 42 g/L, and 5 g/L, respectively.

The addition of 300 g/L of dry cells before conventional operation should have increased the fermentor cell concentration to over 60 g/L; however, it actually increased to 50 g/L. This discrepancy could have been

FIGURE 5-2

FERMENTOR CONDITIONS
DURING HYDROLYZATE RUN
(With Additional Salts)



caused by binding agents and other non-cellular material present in the dry yeast.

First Extractive Fermentation Period (23.0 - 49.0 hrs)

At 23.0 hrs the system was switched to Extractive Fermentation. The aqueous dilution rate was decreased to 0.15 h^{-1} while solvent flow was started at 1.25 h^{-1}. The medium consisted of undiluted hydrolyzate plus nutrients, and was at an average concentration of 335 g/L glucose. Steady state was achieved by 35.0 hours. Mean steady state conditions were 52 g/L ethanol, 46 g/L cells, and 15 g/L residual glucose.

Second Extractive Fermentation Period (49.0 - 76.5 hrs)

At 49.0 hrs the aqueous dilution rate was increased to 0.18 h^{-1}. During this period there was some difficulty in centrifuge operation. Cells were observed in the centrifuge solvent discharge stream at 53.5 hours. A mass of cells had accumulated in the cell discharge tray due to the high viscosity of the cell/solvent mixture, and these in turn appeared to be pushing up into the solvent tray. The centrifuge was subsequently cleaned and the centrifuge discharge time reduced from 15 minutes to 10 minutes. The centrifuge rpm was also increased from 5000 to 6000 rpm, and approximately 78 g of cells were removed from the system. At 69.0 hours yeast cells were observed in the centrifuge operating water drain line. Upon inspection it was found that the centrifuge cell discharge tray was again full of cells and solvent and that the mixture was overflowing into the drain line. The tray was cleaned, and the cell mass in the tray was removed from the system. These activities caused the cell concentration to fluctuate as shown in Figure 5-2. The ethanol concentration meanwhile, decreased steadily over this period, reaching a final concentration of 43 g/L. Residual glucose increased slowly and finally stabilized at 39 g/L.

Third Extractive Fermentation Period (76.5 - 96.0 hrs)

At the beginning of the period the solvent dilution rate was increased to 1.48 h^{-1}, while the aqueous dilution rate remained at 0.18 h^{-1}. There was an initial decrease in cell concentration from 65 g/L to 30 g/L over an eight hour period, which could have been caused by the extensive cell removal from the previous period. The cell concentration eventually stabilized at 30 g/L, while ethanol and residual glucose were 44 g/L and 65 g/L on average. No cells were removed from the centrifuge during this period.

Fourth Extractive Fermentation Period (96.0 - 119.0 hrs)

The purpose of the last Extractive Fermentation period was to observe the effects of using CASCO hydrolyzate without added salts. The medium was switched to straight hydrolyzate at 96.0 hours. No other changes were made to the system. As shown in Figure 5-2, there was a dramatic increase in glucose concentration following the change. The final concentration was 169 g/L at 119.0 hours, while ethanol and cells were at 37 g/L and 42 g/L, respectively. The system was subsequently shut down.

To summarize, the mean steady state fermentor conditions during each period are presented in Table 5-3.

TABLE 5-3

Mean Fermentor Conditions During Hydrolyzate Run

Period		Ethanol (g/L)	Live Cells (g/L)	Residual Glucose (g/L)
Conventional	(0-23 hrs)	54	42	5
First EF	(23-49 hrs)	52	46	15
Second EF	(49-76.5 hrs)	43	46	39
Third EF	(76.5-96 hrs)	44	30	65
Fourth EF	(96-119 hrs)	37	42	169

5.2.3 Ethanol Production

The mean ethanol productivities observed over the run are presented in Table 5-4. The total productivity was approximately equal during each Extractive Fermentation period; thus, increasing the aqueous and solvent dilution rates at the fermentor cell concentrations present had no effect on productivity, due to the lower ethanol concentrations encountered at higher flowrates.

TABLE 5-4

Ethanol Productivities During Hydrolyzate Run

Period		Aqueous (g/L·h)	Solvent (g/L·h)	Total (g/L·h)
Conventional	(0-23 hrs)	10.7	0	10.7
First EF	(23-49 hrs)	7.8	11.5	19.2
Second EF	(49-76.5 hrs)	7.7	10.4	18.1
Third EF	(76.5-96 hrs)	7.8	12.3	20.1
Fourth EF	(96-119 hrs)	6.7	12.3	18.9

A total of 2490 g of ethanol was collected in the condensate during the run. Based on the amount of ethanol leaving in the flashed solvent stream, approximately 50% of the ethanol was not recovered. During this run (as with all runs during the summer months) the condensor was cooled by chilled ethylene glycol instead of cooling water. The coolant was circulated though the condensor by means of a peristaltic pump, while temperature was controlled by operating the fluid cooling unit on a timer. This system presented some difficulties as the temperature could not fall below 0 °C (or else the pump tubing could crack due to ice formation) and yet had to remain below 10 °C to provide adequate ethanol recovery. During this run the coolant temperature was between 7 - 15 °C; the ethylene glycol cooler was set to 40 minutes on/20 minutes off. The mean mass-based ethanol distribution coefficient was 0.23 .

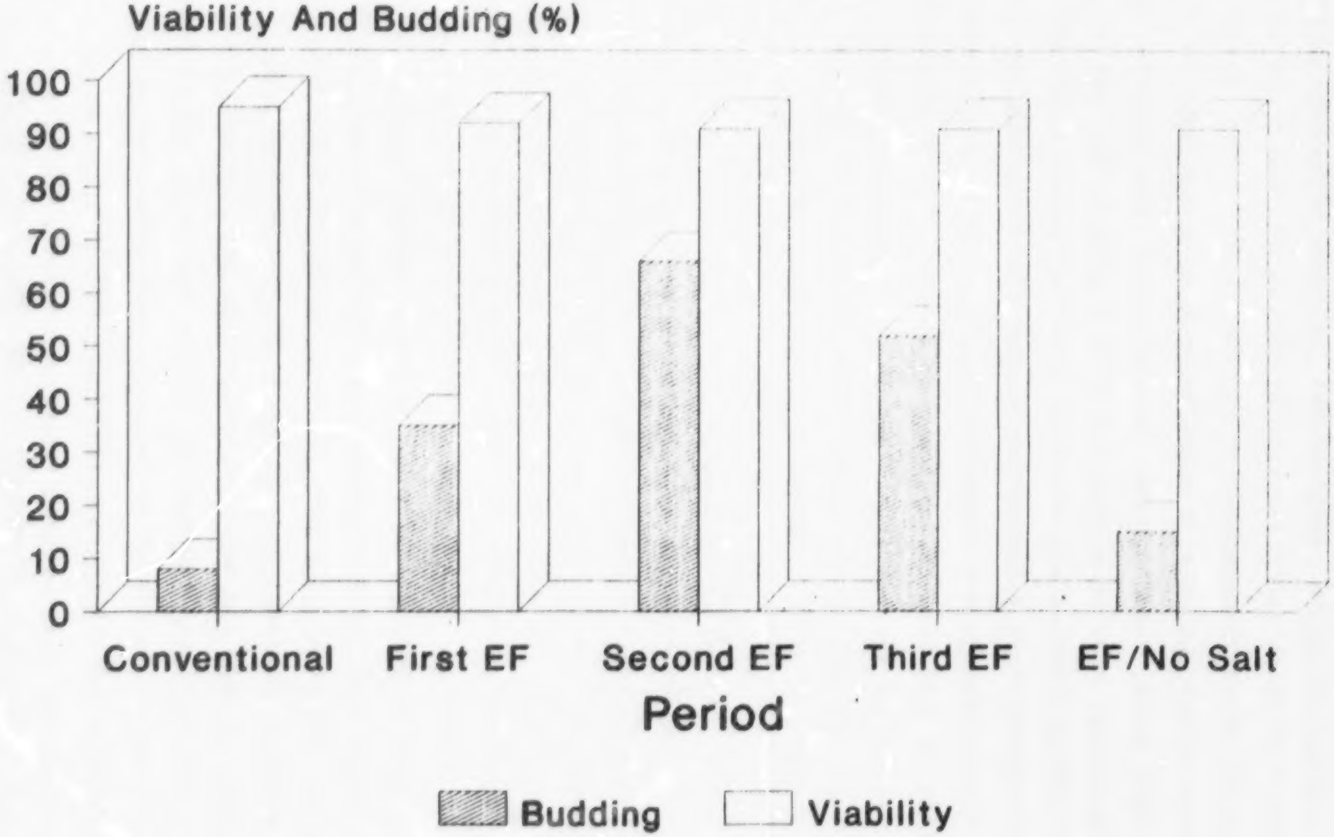
5.2.4 Cell Viability and Budding

The observed cell viability and budding during the various run periods are presented in Figure 5-3. As shown, cell budding was generally higher during Extractive Fermentation as compared to conventional fermentation. This phenomenon has been observed in other PDU runs. Cell budding at the end of the conventional period was only at 8%, while it reached a maximum of 66% during the second Extractive Fermentation period. Budding was, however, considerably lower during the final Extractive Fermentation period compared to the other thee periods. At the end of the run, budding was at 15%, compared to 52% during the third Extractive Fermentation period. Clearly the straight hydrolyzate medium was lacking in some nutrient(s) which are essential for growth. Conversely, cell viability was virtually constant

FIGURE 5-3

CELL VIABILITY AND BUDDING

During Hydrolyzate Run



during the entire run, and was unaffected by the change to the no-salt medium. Over the course of the run, cell viability never fell below 89%.

5.2.5 Cell Production

After the initial inoculation with dried yeast, no cells were added to the system. Although the fermentor cell concentration appeared relatively constant, there was evidence of cell production, both though the high budding observed and cells which had accumulated in the centrifuge. In two instances during Extractive Fermentation operation, the cell return tray in the centrifuge was completely full with a thick cell/solvent mixture, which was removed from the system. Cells were also occasionally observed in the centrifuge solvent stream and in the centrifuge process water drain line. Thus, a considerable amount of cells were both produced and removed from the system.

5.2.6 Discussion of Results

The hydrolyzate appeared to be an excellent substrate for Extractive Fermentation. Conversion during the first Extractive Fermentation period was about 96%, while there was evidence of increased cell production as already mentioned. As well, total ethanol productivity during the Extractive Fermentation periods was almost double that of the conventional period. It is noteworthy to add that, compared to previous runs, this run provided some of the highest measurements of cell viability. This may have been due to the presence of trace nutrients not properly supplied in synthetic medium.

The medium did have to be supplemented with salts and yeast extract, however, because the particular type of hydrolyzate supplied by CASCO Inc. is used for dextrose production, and thus has most of the proteins and other nutrients removed, as well as all salts and solids. An industrial Extractive Fermentation facility would likely use a less refined hydrolyzate which would not require additional nutrients.

6.0 EXTRACTIVE FERMENTATION WITH ZYMOMONAS MOBILIS

The organism *Zymomonas mobilis* has been shown to be an attractive alternative to yeast fermentation due to its ability to tolerate high ethanol and sugar concentrations and its higher ethanol productivity (Rogers, et al., 1982). Recent work has also revealed it may be a useful organism for molasses fermentation (Doelle and Doelle, 1990). These characteristics, combined with improved fermentation capabilities though Extractive Fermentation, would make for a very efficient ethanol production system.

The PDU was tested with *Z. mobilis* to explore the possibilities of combining this organism's superior abilities with Extractive Fermentation technology. Earlier tests had shown that the continuous centrifuge would adequately separate the organism from the solvent and the aqueous phases, thus allowing for cell recycle. The substrate was a synthetic medium with glucose as a carbon source, similar to that used for yeast fermentation; the concentrations of salts and yeast extract in the medium, which were independent of the glucose concentration, are presented in Table 6-1.

TABLE 6-1

Medium Formulation for Zymomonas Mobilis

Nutrient	Amount Added
$(NH_4)_2SO_4$	1.94 g/L
KH_2PO_4	7.76 g/L
$MgSO_4 \cdot 7H_2O$	0.97 g/L
Yeast Extract	17.00 g/L

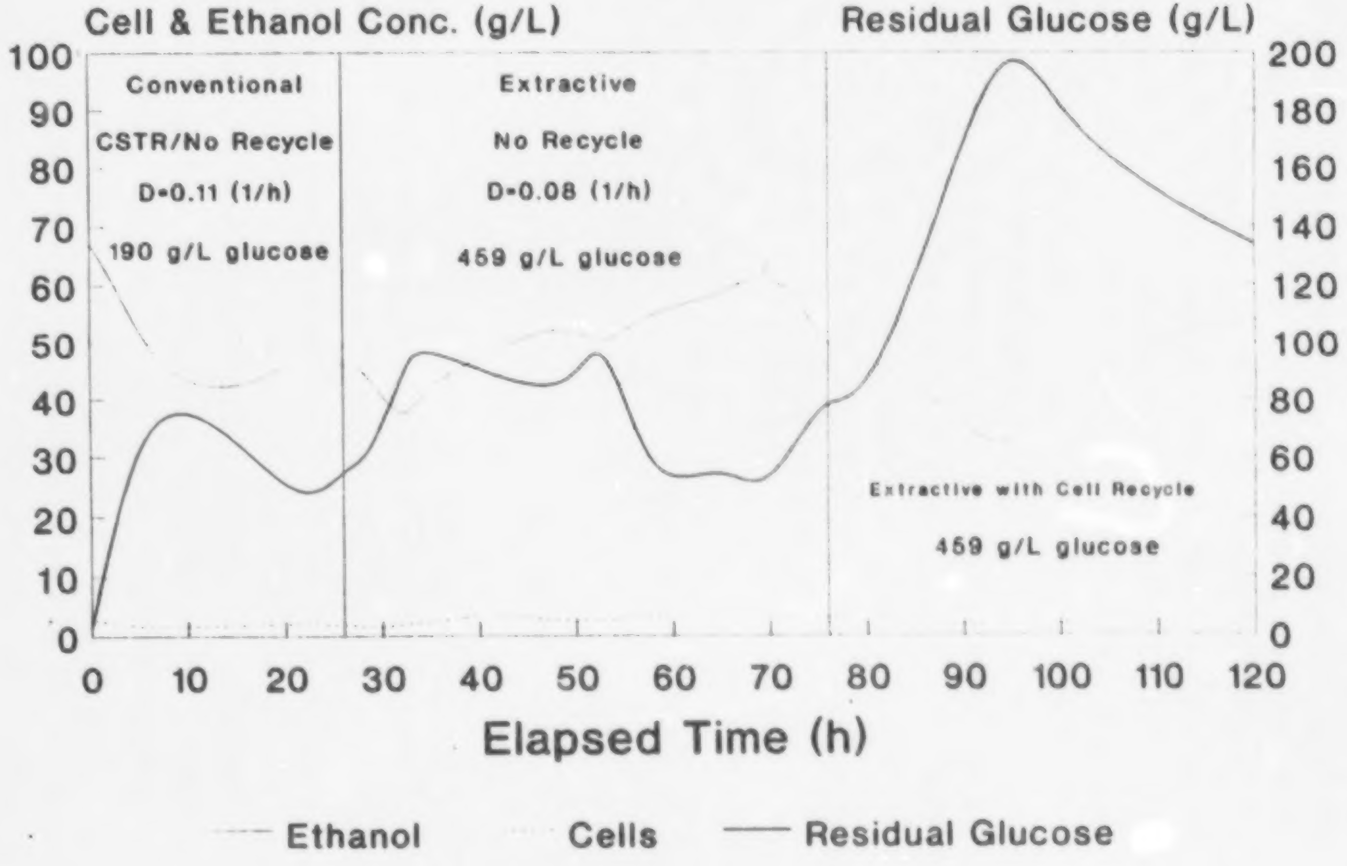
6.1 FERMENTOR CONDITIONS

After an initial 24 hour period of batch growth conventional operation with cell recycle was initiated. The fermentor was operated under these conditions for 26 hours. The pH was controlled at about 5.1. During this period the aqueous dilution rate was 0.11 h^{-1}, while the medium concentration was 195 g/L glucose. At the end of this period ethanol was at 47 g/L, cell concentration at 1.9 g/L, and residual glucose at 57 g/L. Fermentor conditions are shown in Figure 6-1.

At 26.0 hours Extractive Fermentation was begun. The aqueous dilution rate was set at 0.08 h^{-1} while the solvent dilution rate was approximately 1.25 h^{-1}. The medium was at 190 g/L glucose for the first 3.5 hours, after which it was switched to 440 g/L glucose (average concentration after this switch was 459 g/L). No cells were returned to the fermentor, although about 400 mL of "spent" fermentation broth were returned at 56.5

FIGURE 6-1

FERMENTOR CONDITIONS
DURING *ZYMOMONAS MOBILIS* RUN



hours to compensate for a loss of fermentor aqueous volume. Average conditions during the last 8 hours of this period were 56 g/L ethanol, 3.2 g/L cells, and 65 g/L residual glucose.

At 76.0 hours cell recycle was begun to both increase the cell concentration and maintain the fermentor aqueous volume. Shortly after, a large increase in residual glucose was observed, as well as a decrease in both ethanol and cell concentrations. By 97.5 hours residual glucose had reached 199 g/L, while ethanol and cell concentrations were at 36 g/L and 1.4 g/L, respectively. It was also observed that the cells had become filamentous. At 97.5 hours batch operation was begun; however, over a period of 22.5 hours glucose decreased to only 134 g/L, while ethanol and cells increased to 54 g/L and 2.4 g/L. There was little evolution of CO_2 observed during batch operation. The system was subsequently shut down.

6.2 ETHANOL PRODUCTION

During conventional operation a total of 635 g of ethanol was produced. The ethanol yield based on substrate consumption was 0.35 g ethanol/g glucose.

During the Extractive Fermentation period without cell recycle a total of 1881 g of ethanol were produced, while 4592 g of glucose were consumed. Thus, the ethanol yield based on substrate was 0.41 g ethanol/g glucose. A total of 1856 g of ethanol was collected in the condensate. Based on the amount of ethanol leaving the solvent, approximately 12% was not recovered. The ethanol concentration in the condensate was on average 46% (wt) and reached a maximum of 58% (wt).

Mean total ethanol productivities during conventional and Extractive Fermentation without cell recycle were 4.8 g/L·h and 10.6 g/L·h, respectively.

6.3 CELL PRODUCTION

During conventional fermentation a total of 23.6 g of cells left the system, while a total of 1808 g of glucose were utilized. Thus, the cell yield was 0.013 g cells/g glucose. During Extractive Fermentation operation without cell recycle a total of 37.3 g of cells left the fermentor while 4592 g of glucose were used, providing a cell yield of 0.008 g cells/g glucose. It is important to note that the cell yield during Extractive Fermentation was based on the mass of cells leaving in the aqueous stream. The amount of cells entrained in the solvent stream was not measured; thus, the calculated cell yield could be considerably higher.

6.4 DISCUSSION OF RESULTS

The results showed great promise compared to yeast. For example, the ethanol yield based on ZM cells (g ethanol/g cells) was 27.0 g ethanol/g cells, while a "typical" yield for *Saccharomyces Cerevisiae* would be 7.6 g ethanol/g cells (Kollerup, 1986). Thus higher ethanol productivities can be obtained without the high cell concentrations required for yeast-based fermentations.

The organism appeared to be sensitive to stillage recycle, possibly because of the accumulation of salts. Similar declines in cell activity have been experienced with yeast during Extractive Fermentation and were solved by reducing the amount of salts in the medium as already mentioned. Other work (Iida, 1990) has shown that some trace elements, such as copper, are very inhibitory and that the maximum allowable salt concentration (as ash, primarily calcium and magnesium) for the organism is about 1.7% (wt). It has also been shown that molasses fermentation by *Z. mobilis* can be significantly improved if the salts are first removed. It may be possible to remove most of the inhibitory salts with inexpensive sulfuric acid.

7.0 TECHNOLOGY TRANSFER

The final goal of the Extractive Fermentation project is transfer of the process to the private sector. Activities directed towards this goal have been greatly assisted by PARTEQ, an organization within Queen's University designated to facilitate the transfer of technology to private industry. With their assistance, a licensing agreement is currently being discussed with Monsanto Enviro-Chem, a wholly-owned subsidiary of Monsanto Corporation (St. Louis, MO) and JonTon Alcohol, a fermentation ethanol company in Edinburg, Texas. The agreement deals with the retrofit of the JonTon plant with Extractive Fermentation technology; Monsanto is involved with the final design and costing of the facility. North American companies have shown interest and been involved in the Extractive Fermentation project and the prospects of commercialization. The most notable of these are:

A. E. Staley
Grain Processing Corporation
Archer Daniels Midland
RockCliffe Technologies
Commercial Alcohols
Mohawk Fuels
Raphael Katzen and Associates
Vogelbusch
U. S. Department of Energy
Alcohol Producers Inc.
Wardrop Engineering

Several Japanese firms have also expressed considerable interest in the project. Representatives from Suntory Corporation Ltd. (Japan) have visited twice to view the project and discuss technology acquisition. Both NEDO (New Energy and Industrial Technology Development Organization) and FARA (Fuel Alcohol Resources Association) have also become involved. With the recent uncertainty of foreign energy supplies, both domestic and foreign interest in the project is expected to increase.

The Extractive Fermentation business plan is presented in Appendix F.

8.0 FUTURE WORK

The development of Extractive Fermentation technology had begun with the mathematical modeling of the process concept (Kollerup and Daugulis, 1985a), and has proceeded though a systematic search for an appropriate extracting solvent (Kollerup and Daugulis, 1985b; Kollerup and Daugulis, 1986), prototype development (Kollerup and Daugulis, 1987), process demonstration (Daugulis, *et al.*, 1987), and finally process optimization and economic analysis, which have been presented in this report. The operation of the upgraded Extractive Fermentation Process Demonstration Unit represents the last stage in laboratory scale research. The process has been demonstrated using equipment virtually identical, although on a smaller scale, to that used in an industrial-scale system.

The next logical step is a pilot-scale study of the process. Pilot-scale operation would not only confirm the benefits of the process on a larger scale, but would also overcome some of the inherent difficulties encountered on the laboratory scale. Some of these difficulties arose because the proper equipment size was not available. The centrifuge, for example, was oversized for the process flowrates and was not the proper type for some of the cell concentrations used (Alfa-Laval, 1979). The vacuum flash unit was also oversized to accommodate the sight glasses mounted on the sides. Other difficulties were caused because of the low flowrates involved. It was found, for example, that a small-bore spray nozzle could not be used in the vacuum flash unit because it was prone to clogging; however, a larger-sized nozzle could not be used because of the low solvent flowrate. The low flowrates also required the use of peristaltic pumps, which were susceptible to collapsed and split tubing. These difficulties would be solved in a pilot-scale operation.

More importantly, however, is the testing of Extractive Fermentation on a larger scale. There are numerous unknowns concerning larger scale operation, such as the need for solvent recycle, the effect of high cell concentrations, the aeration rate required, the degree of aqueous and cell entrainment in the solvent, and flash unit and centrifuge performance. It is also believed that performance of the oversized equipment will improve with increased scale. These questions and others must be addressed before any industrial-scale commitment is made.

The estimated size and cost of a pilot plant facility is presented in Table 8-1. The costs are based on 1990 quotes from suppliers where possible and are expressed in Canadian dollars. The flash drum size is assumed to have the same volume as our current unit. The fermentor cost is based on a quote from a supplier (Alfa-Laval Ltd., Scarborough, Ont.). It is noteworthy to add that much of the equipment required (centrifuge, flash unit, several pumps) is already part of the current PDU and could successfully be used for a pilot scale operation since it is oversized for the current system. Also, Alfa-Laval Canada Ltd. (Scarborough, Ontario) has agreed to donate a heat exchanger for the project.

Natural substrates are another area of interest requiring further work. Although the hydrolyzate run provided excellent results, there were difficulties with molasses. It is imperative that these areas be addressed before the JonTon retrofit scheme commences, since their facility uses blackstrap molasses as a substrate for fermentation. Other companies have overcome similar problems with feedstocks (such as Alfa-Laval with their Biostil® process) but only after intensive research.

TABLE 8-1

Estimated Equipment Costs For Extractive Fermentation Pilot Plant

EQUIPMENT	SIZE	COST
Fermentor	150 L	$140,000
Centrifuge	200 L/h	$ 50,000
Flash Unit	10 L	$ 1,600
Aqueous Pumps (4)	20 L/h	$ 8,000
Solvent Pumps (5)	200 L/h	$ 25,000
Surge/Storage Vessels	600 L	$ 4,500
Heat Exchanger	200 L/h	$ 1,000
Control System		$ 18,300
Fittings/Valves		$ 15,000
Total Equipment Cost		$263,400

The prospects for *Z. mobilis* are encouraging. This organism does appear to have a great capacity for ethanol production; however, both ethanol production and specific growth rate can be severely inhibited by ethanol concentrations in the neighbourhood of 60 g/L (Lee, et al., 1980). It would therefore appear to be an ideal situation where Extractive Fermentation could be applied. More research is needed in this area as well, however, since several other factors, such as stillage recycle, seem to have a tremendous effect on cell activity.

The merging of Extractive Fermentation to molecular sieve technology could prove to be an extremely economical method of producing ethanol. Recent work has shown that anhydrous ethanol can be produced without distillation by using relatively inexpensive adsorbents, such as corn grits (Lee and Ladisch, 1987). The only drawback is that such an adsorption column requires both that the feed stream contains a high concentration of ethanol (>50% wt), and that it is in the vapour phase. These conditions are met precisely by the Extractive Fermentation flash unit. By mating the two technologies, it is conceivably possible to produce anhydrous ethanol without distillation. Further research in this direction should be undertaken.

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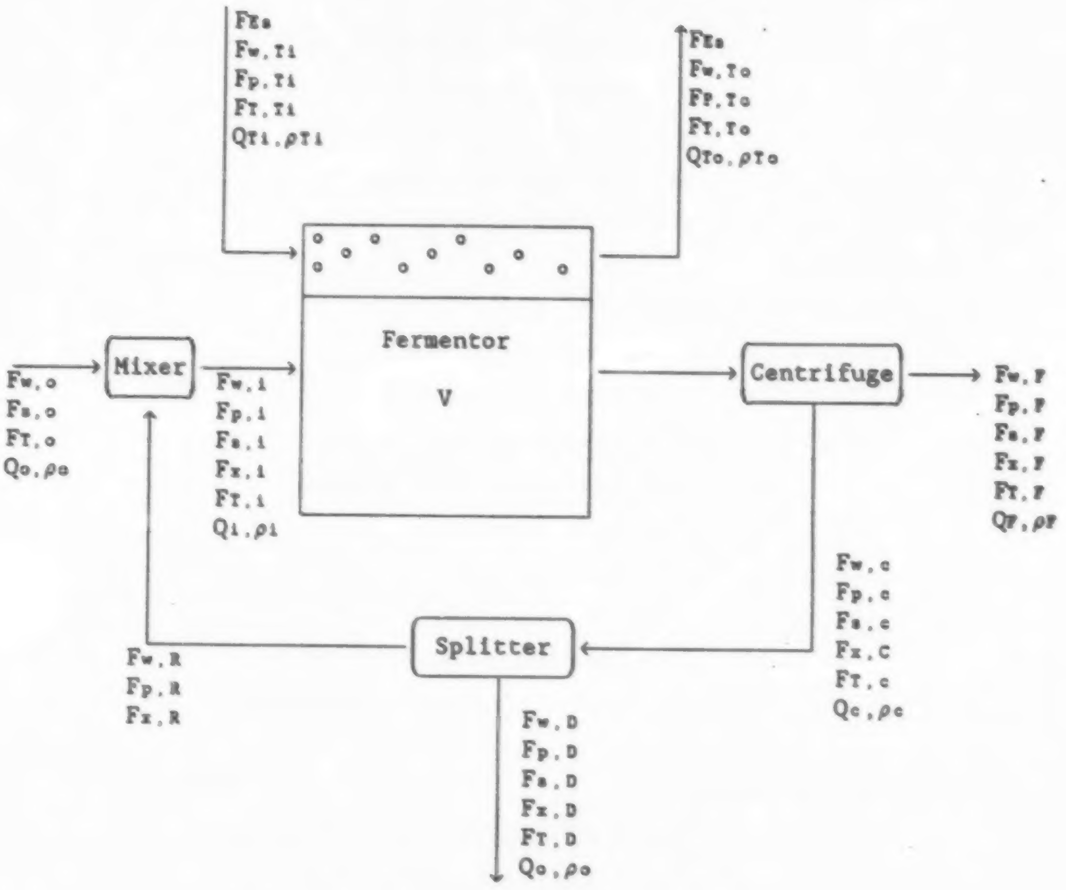
APPENDIX A
MATHEMATICAL MODELLING

The fermentor model used in PROCESS^{TM} was based on a rigorous mass balance around the fermentor and recycle apparatus. The system, illustrated below, and the nomenclature used in describing it follows.



F_{Es}
$F_{w,Ti}$
$F_{p,Ti}$
$F_{T,Ti}$
Q_{Ti}, ρ_{Ti}
F_{Es}
$F_{w,To}$
$F_{P,To}$
$F_{T,To}$
Q_{To}, ρ_{To}
Fermentor
V
$F_{w,o}$
$F_{s,o}$
$F_{T,o}$
Q_o, ρ_o
Mixer
$F_{w,i}$
$F_{p,i}$
$F_{s,i}$
$F_{x,i}$
$F_{T,i}$
Q_i, ρ_i
Centrifuge
$F_{w,F}$
$F_{p,F}$
$F_{s,F}$
$F_{x,F}$
$F_{T,F}$
Q_F, ρ_F
$F_{w,c}$
$F_{p,c}$
$F_{s,c}$
$F_{x,C}$
$F_{T,c}$
Q_c, ρ_c
Splitter
$F_{w,R}$
$F_{p,R}$
$F_{x,R}$
$F_{w,D}$
$F_{p,D}$
$F_{s,D}$
$F_{x,D}$
$F_{T,D}$
Q_D, ρ_D

NOMENCLATURE

F = Mass Flowrate (kg/h)

Q = Volumetric Flowrate (m^3/h)

ρ = Stream Density (kg/m^3)

V = Reactor Volume (m^3)

Material Subscripts:

w = water

s = substrate (fermentables)

p = product (ethanol)

x = cells

Es = extracting solvent

CO_2 = carbon dioxide

Stream Subscripts:

o = fermentables feed

i = inlet to reactor

F = centrifuge supernatant

c = centrifuge yeast stream

D = dryer feed

R = recycle

Ti = solvent ("top") inlet

To = solvent ("top") outlet

T = total of stream components

Additional Variables:

$Y_{p/s}$ = ethanol yield coefficient

$Y_{c/s}$ = CO_2 yield coefficient

β = ethanol distribution coefficient

μ = specific growth rate (h^{-1})

μ_{max} = maximum specific growth rate (h^{-1})

K_p = ethanol inhibition constant (kg/m^3)

Ks = substrate saturation constant (kg/m^3)

γ = mass ratio (water/ethanol) in solvent outlet stream

b_b = cell mass fraction in centrifuge yeast stream

Rr = recycle ratio (for splitter)

α_1, α_2 = coefficients for aqueous stream density

MASS BALANCES

I Fermentor

(1) Ethanol Balance

$$F_{p,i} + Y_{p/s}(F_{s,i} - F_s) - F_p - F_{p,To} + F_{p,Ti} = 0 \tag{1}$$

Additional Relationship:

$$F_{p,To} = \frac{\beta(F_p/Q)(F_{Es}/\rho_{Es})}{1 - \beta(F_p/(Q \cdot \rho_p))}$$

$$F_{p,To} = \frac{\beta\ F_p\ (F_{Es}/\rho_{Es})}{Q - \beta\ (p/\rho_p)} \tag{2}$$

(2) Cell Balance

$$F_{x,i} + V\ \mu\ (F_x/Q) - F_x = 0 \tag{3}$$

Additional Relationship:

$$\mu = \mu_{max} \frac{(F_s/Q)}{(F_s/Q + K_s)} \left[1 - \frac{(F_p/Q)}{P_{max}} \right]^n \tag{4}$$

(3) CO_2 Balance

$$Y_{c/s}\ (F_{s,i} - F_s) - F_{co2} = 0 \tag{5}$$

(4) Water Balance

$$F_{w,i} + F_{w,Ti} = F_w + F_{w,To} \tag{6}$$

Additional Relationship:

$$F_{w,To} = \gamma\ F_{p,To} \tag{7}$$

(5) Overall Balance

$$F_{T,i} + F_{w,Ti} + F_{p,Ti} - F_T - F_{w,To} - F_{p,To} - F_{co2} = 0 \tag{8}$$

(6) Stream Relationships

$$F_T = F_w + F_s + F_p + F_x \tag{9}$$

Additional Relationship:

$$\rho = 1000 + \alpha_1 (F_s/Q) + \alpha_2 (F_p/Q)$$

and

$$Q = F_T/\rho$$

$$\therefore \quad Q = (F_T - \alpha_1 F_s - \alpha_2 F_p) / 1000 \qquad (10)$$

II Centrifuge

(1) Cell Balance

$$F_{x,c} = F_x \quad ; \quad F_{x,F} = 0 \quad \text{(assuming 100\% cell separation)}$$

(2) Concentration Factor

$$b_b = F_{x,c}/F_{T,c}$$

$$\therefore \quad F_{T,c} = F_x/b_b$$

(3) Aqueous Stream

$$\rho = \rho_c = \rho_F \quad \text{(density assumed unchanged)}$$

$$F_{T,c} = Q_c \, \rho_c$$

$$\therefore \quad Q_c = F_x/(\rho \, b_b)$$

Also

$$F_{s,c}/Q_c = F_s/Q \quad \text{(density assumed unchanged)}$$

$$\therefore \quad F_{s,c} = \frac{F_x \, F_s}{\rho \, Q \, b_b} = \frac{F_x \, F_s}{F_T \, b_b}$$

Similarly,

$$F_{p,c} = \frac{F_x \, F_p}{F_T \, b_b}$$

Also

$$F_{w,c} = F_{T,c} - F_x - F_{s,c} - F_{p,c}$$

$$\therefore \quad F_{w,c} = F_x \left[1/b_b - 1 - F_p/(F_T \, b_b) - F_s/(F_T \, b_b) \right]$$

Also

$$F_{i,F} = F_i - F_{i,c} \quad \text{for each species}$$

III Splitter

(1) Recycle Ratio Definition

$$F_{T,R} = R_r \, F_{T,c}$$

$$\therefore \quad F_{T,R} = R_r \, (F_x/b_b)$$

$$F_{p,R} = Rr\ F_{p,c} = Rr \left[\frac{F_x\ F_p}{bb\ F_T} \right]$$

$$F_{s,R} = Rr\ F_{s,c} = Rr \left[\frac{F_x\ F_s}{bb\ F_T} \right]$$

$$F_{x,R} = Rr\ F_{x,c} = Rr\ F_x$$

$$F_{w,R} = Rr\ F_{w,c} = Rr\ F_x \left[\frac{1}{bb} - 1 - \frac{F_p}{bb\ F_T} - \frac{F_s}{bb\ F_T} \right]$$

Also, for the dryer feed stream:

$F_{i,D} = (1\text{-}Rr)\ F_{i,c}$ for each species

IV Mixer

$$F_{p,i} = F_{p,R} = \frac{Rr\ F_x\ F_p}{bb\ F_T}$$

$$F_{s,i} = F_{s,o} + F_{s,R} = F_{s,o} + \frac{Rr\ F_x\ F_s}{bb\ F_T}$$

$$F_{x,i} = F_{x,R} = Rr\ F_x$$

$$F_{w,i} = F_{w,o} + F_{w,R} = F_{w,o} + Rr\ F_x \left[\frac{1}{bb} - 1 - \frac{F_p}{bb\ F_T} - \frac{F_s}{bb\ F_T} \right]$$

$$F_{T,i} = F_{s,o} + F_{w,o} + (Rr\ F_x)/bb$$

There are 25 mass balance equations and 26 unknowns; therefore, one variable had to be specified. The fermentor outlet cell concentration was, therefore, specified:

$C_x = F_x/Q$ where C_x is known

Additional calculation:

Fermentor Heat Generation = $(0.1678)(F_{s,i} - F_s)(4.186)$ kJ/h

Solution Strategy

The 25 equations could have been solved directly; however, the appropriate substitutions were made to allow the reactor to be solved first. This resulted in 5 reactor equations:

From Mixer equations and equations (1) and (2):

$$\frac{R_r\ C_x\ Q\ F_p}{b_b\ F_T} + Y_{p/s}\left[\ F_{s,o} + \frac{R_r\ C_x\ Q\ F_s}{b_b\ F_T} - F_s\ \right] - F_p - \frac{\beta\ F_p\ (F_{Es}/\rho_{Es})}{Q - \beta\ (F_p/\rho_p)} + F_{p,Ti} = 0$$

From equations (3) and (4):

$$Q\ (R_r - 1) + \frac{V\ \mu_{max}\ F_s}{F_s + K_s\ Q}\left[\ 1 - \frac{F_p}{P_{max}\ Q}\ \right]^n = 0$$

From mixer equations (5), (6), (7), and (8):

$$F_{p,Ti} + F_{w,Ti} + F_{s,o} + F_{w,o} + \frac{R\ C_x\ Q}{b_b} - F_T - (1-\gamma)\frac{\beta\ F_p\ (F_{Es}/\rho_{Es})}{Q - \beta(F_p/\rho_p)}$$
$$- Y_{c/s}\left[F_{s,o} + \frac{R\ C_x\ Q\ F_s}{b_b} - F_s\right] = 0$$

From mixer equations (6) and (9):

$$F_T - F_{w,Ti} + \frac{\gamma\ \beta\ F_p(F_{Es}/\rho_{Es})}{Q - \beta(F_p/\rho_p)} - F_{w,o} - R\ C_x\ Q\left[\frac{1}{b_b} - 1 - \frac{(F_p + F_s)}{b_b\ F_T}\right]$$
$$- F_s - F_p - C_x\ Q = 0$$

From equation (10):

$$Q = \frac{F_T - \alpha_1\ F_s - \alpha_2\ F_p}{1000}$$

There are 5 equations to be solved. In order to simplify the calculation of partial derivatives, the last equation will be considered "constant". Before the first four equations are calcualted, Q will be found using previous iteration values.

Define:

$x1 = R$
$x2 = F_p$
$x3 = F_s$
$x4 = F_T$

Gives: $Q = (x4 - \alpha_1\ x3 - \alpha_2\ x2) / 1000$

$$f_1 = (Q - \beta\ x2/\rho_p)\left[\frac{C_x\ Q\ x1}{b_b\ x4}(x2 + Y_{p/s}\ x3) + Y_{p/s}\ F_{s,o} - Y_{p/s}\ x3 - x2 + F_{p,Ti}\right] - \beta\ x2\ (F_{Es}/\rho_{Es}) \tag{1}$$

$$f_2 = (k_s\ Q + x3)\ Q\ (x1 - 1) + V\ \mu_{max}\ x3\left[1 - \frac{x2}{Q\ P_{max}}\right]^n \tag{2}$$

$$f_3 = (Q - \beta\ x2 / \rho_p)\left[F_{w,Ti} + F_{p,Ti} + F_{s,o}(1 - Y_{c/s}) + F_{w,o} - x4 + Y_{c/s}\ x3 + \frac{x1\ C_x\ Q}{b_b}(1 - Y_{c/s}\ x3 / x4)\right] - \beta\ x2\ (F_{Es}/\rho_{Es})(1+\gamma) \tag{3}$$

$$f_4 = (Q - \beta\ x2/\rho_p)\left[x4 - F_{w,Ti} - F_{w,o} - C_x\ Q\ x1\left[\frac{1}{b_b} - 1 - (x2+x3)\frac{1}{b_b\ x4}\right] - x2 - x3 - C_x\ Q + \gamma\ \beta\ x2\ (F_{Es}/\rho_{Es})\right. \tag{4}$$

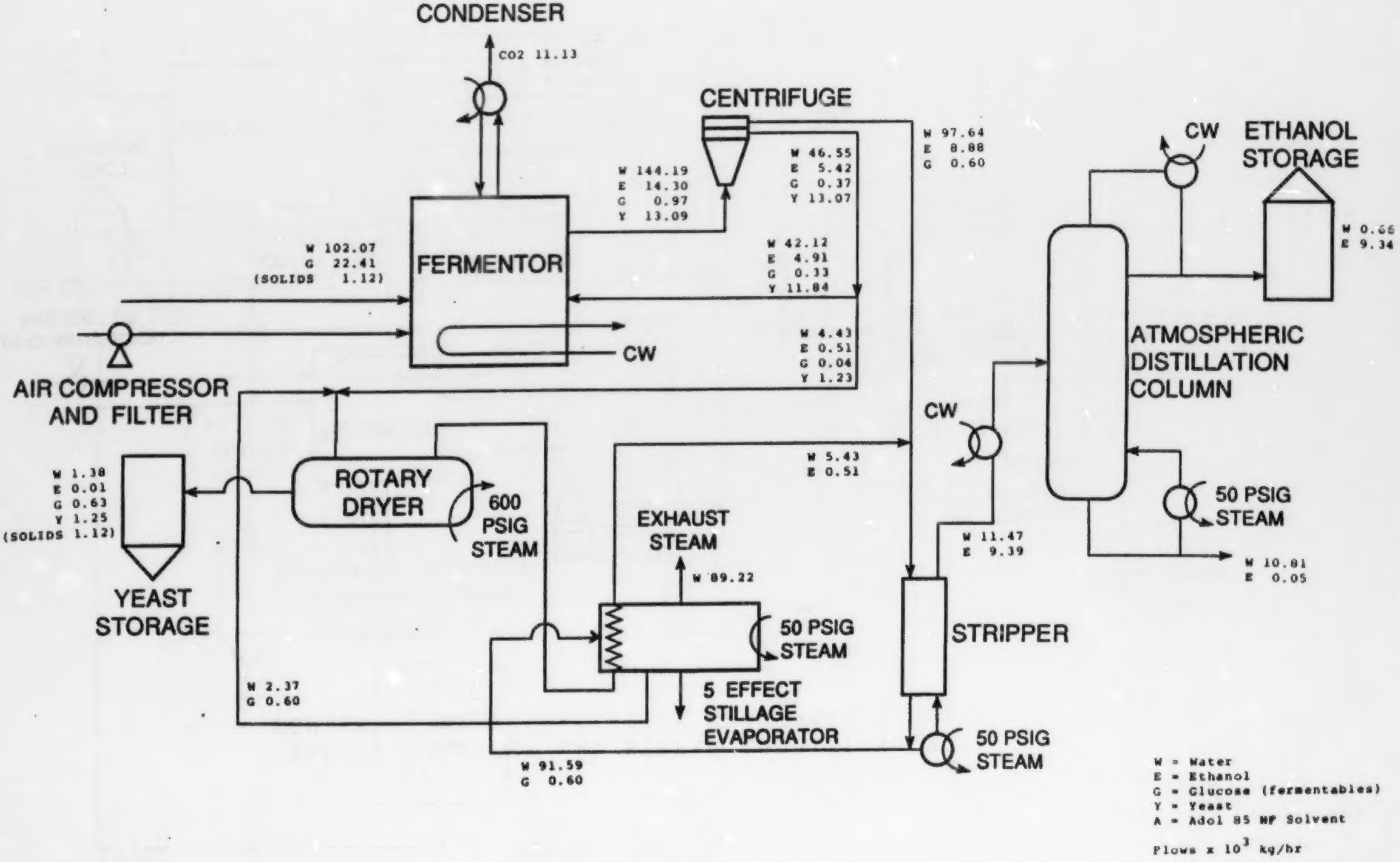
The Newton-Raphson method uses the partial derivatives of equations (1) through (4) with respect to x1, x2, x3, and x4 for solution. Derivation of the partial differential equations are available upon request.

APPENDIX B

MATERIAL AND ENERGY BALANCES FOR CONVENTIONAL AND EXTRACTIVE FERMENTATION PLANTS

The material and energy balances for the grass-roots conventional and Extractive Fermentation plants are provided, as well as balances for the RETRONEW retrofit scenario.

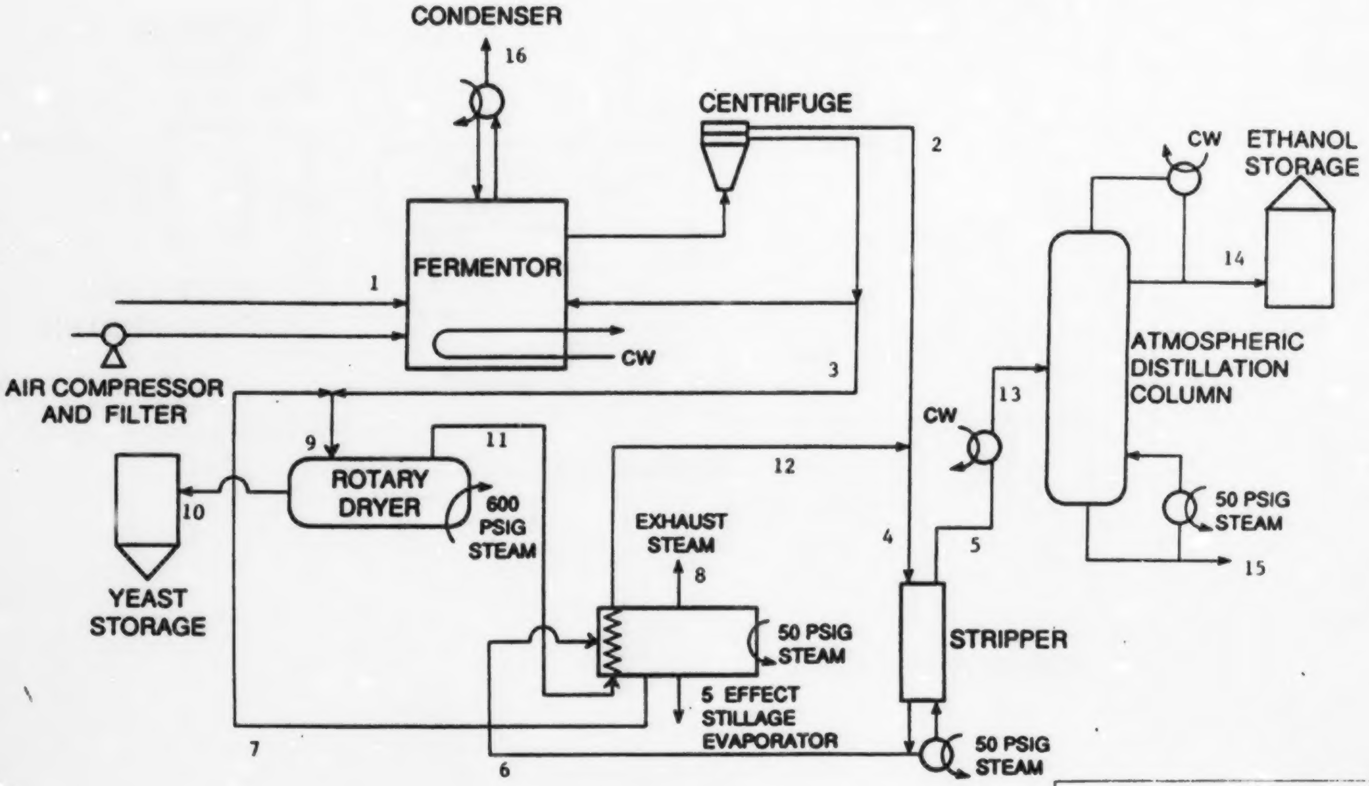
MASS BALANCES FOR OPTIMIZED CONVENTIONAL FERMENTATION GRASS-ROOTS PLANT


CONDENSER
CO2 11.13
CENTRIFUGE
W 144.19
E 14.30
G 0.97
Y 13.09
W 46.55
E 5.42
G 0.37
Y 13.07
W 97.64
E 8.88
G 0.60
CW
ETHANOL STORAGE
W 0.66
E 9.34
W 102.07
G 22.41
(SOLIDS 1.12)
FERMENTOR
W 42.12
E 4.91
G 0.33
Y 11.84
W 4.43
E 0.51
G 0.04
Y 1.23
CW
AIR COMPRESSOR AND FILTER
ATMOSPHERIC DISTILLATION COLUMN
CW
W 5.43
E 0.51
W 1.38
E 0.01
G 0.63
Y 1.25
(SOLIDS 1.12)
ROTARY DRYER
600 PSIG STEAM
EXHAUST STEAM
W 89.22
50 PSIG STEAM
W 11.47
E 9.39
W 10.81
E 0.05
YEAST STORAGE
50 PSIG STEAM
STRIPPER
5 EFFECT STILLAGE EVAPORATOR
W 2.37
G 0.60
W 91.59
G 0.60
50 PSIG STEAM

W = Water
E = Ethanol
G = Glucose (fermentables)
Y = Yeast
A = Adol 85 HF Solvent

Flows x 10^3 kg/hr

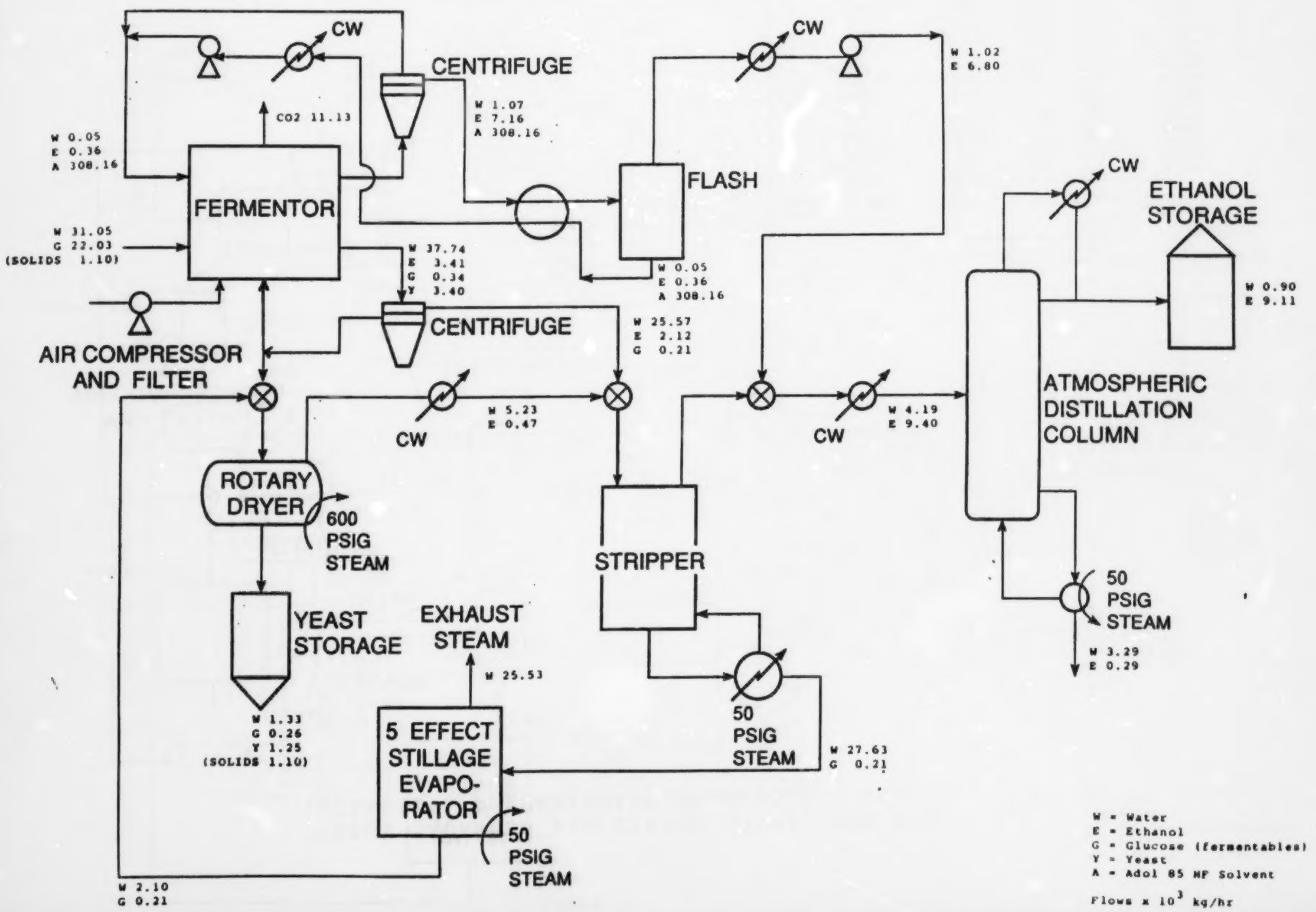
ENERGY BALANCES AND STREAM CONDITIONS FOR CONVENTIONAL FERMENTATION GRASS-ROOTS PLANT


CONDENSER
16
CENTRIFUGE
2
CW
ETHANOL STORAGE
FERMENTOR
1
14
CW
ATMOSPHERIC DISTILLATION COLUMN
AIR COMPRESSOR AND FILTER
3
CW
13
9
11
ROTARY DRYER
12
600 PSIG STEAM
10
EXHAUST STEAM
8
4
5
50 PSIG STEAM
15
YEAST STORAGE
50 PSIG STEAM
STRIPPER
5 EFFECT STILLAGE EVAPORATOR
7
6
50 PSIG STEAM

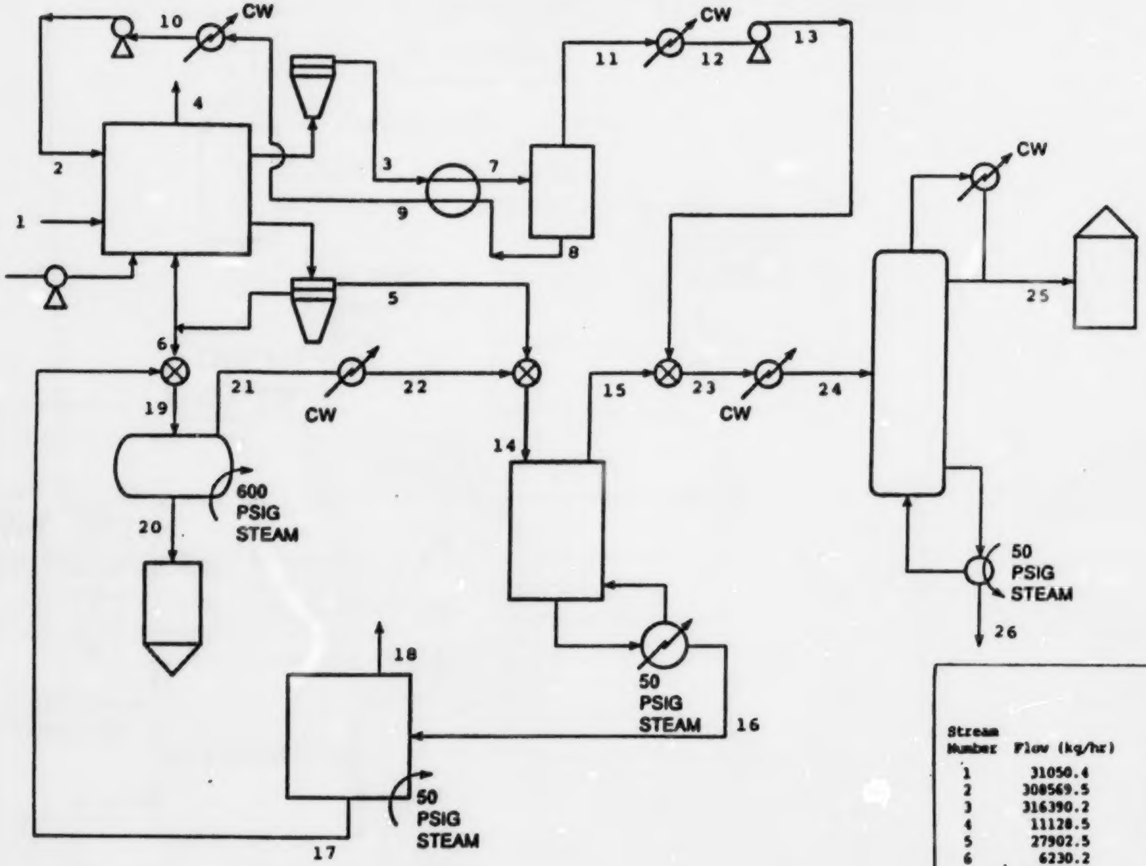
STREAM CONDITIONS
SIMULATION CONV07

Stream Number	Flow (kg/hr)	Phase	T (°C)	P (psia)	Enthalpy (million kJ/hr)
1	102069.4	Liquid	30.0	14.7	
2	107119.3	Liquid	30.0	14.7	3.5646
3	6226.3	Liquid	30.0	14.7	0.2125
4	113052.0	Liquid	34.2	15.5	5.7110
5	20857.2	Vapour	94.2	15.3	40.7501
6	92194.8	Liquid	105.9	18.1	29.8987
7	2973.9	Liquid	106.7	18.1	0.7841
8	89221.3	Vapour	106.7	18.1	239.6761
9	9200.1	Liquid	55.6	25.0	0.9966
10	3267.3	Liquid	114.6	25.0	0.5172
11	5932.8	Vapour	114.6	25.0	15.2171
12	5932.8	Liquid	107.1	25.0	2.1464
13	20857.2	Liquid	96.6	25.0	7.9950
14	10001.5	Liquid	78.7	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

MASS BALANCES FOR OPTIMIZED EXTRACTIVE FERMENTATION GRASS-ROOTS PLANT


CW
CENTRIFUGE
CO2 11.13
W 1.07
E 7.16
A 308.16
W 0.05
E 0.36
A 308.16
FERMENTOR
W 31.05
G 22.03
(SOLIDS 1.10)
W 37.74
E 3.41
G 0.34
Y 3.40
CENTRIFUGE
AIR COMPRESSOR
AND FILTER
CW
W 1.02
E 6.80
FLASH
W 0.05
E 0.36
A 308.16
W 25.57
E 2.12
G 0.21
CW
ETHANOL
STORAGE
W 0.90
E 9.11
W 5.23
E 0.47
CW
CW
W 4.19
E 9.40
ATMOSPHERIC
DISTILLATION
COLUMN
ROTARY
DRYER
600
PSIG
STEAM
STRIPPER
50
PSIG
STEAM
W 3.29
E 0.29
YEAST
STORAGE
EXHAUST
STEAM
W 25.53
W 1.33
G 0.26
Y 1.25
(SOLIDS 1.10)
5 EFFECT
STILLAGE
EVAPO-
RATOR
50
PSIG
STEAM
W 27.63
G 0.21
50
PSIG
STEAM
W 2.10
G 0.21
W = Water
E = Ethanol
G = Glucose (fermentables)
Y = Yeast
A = Adol 85 HF Solvent
Flows x 10³ kg/hr

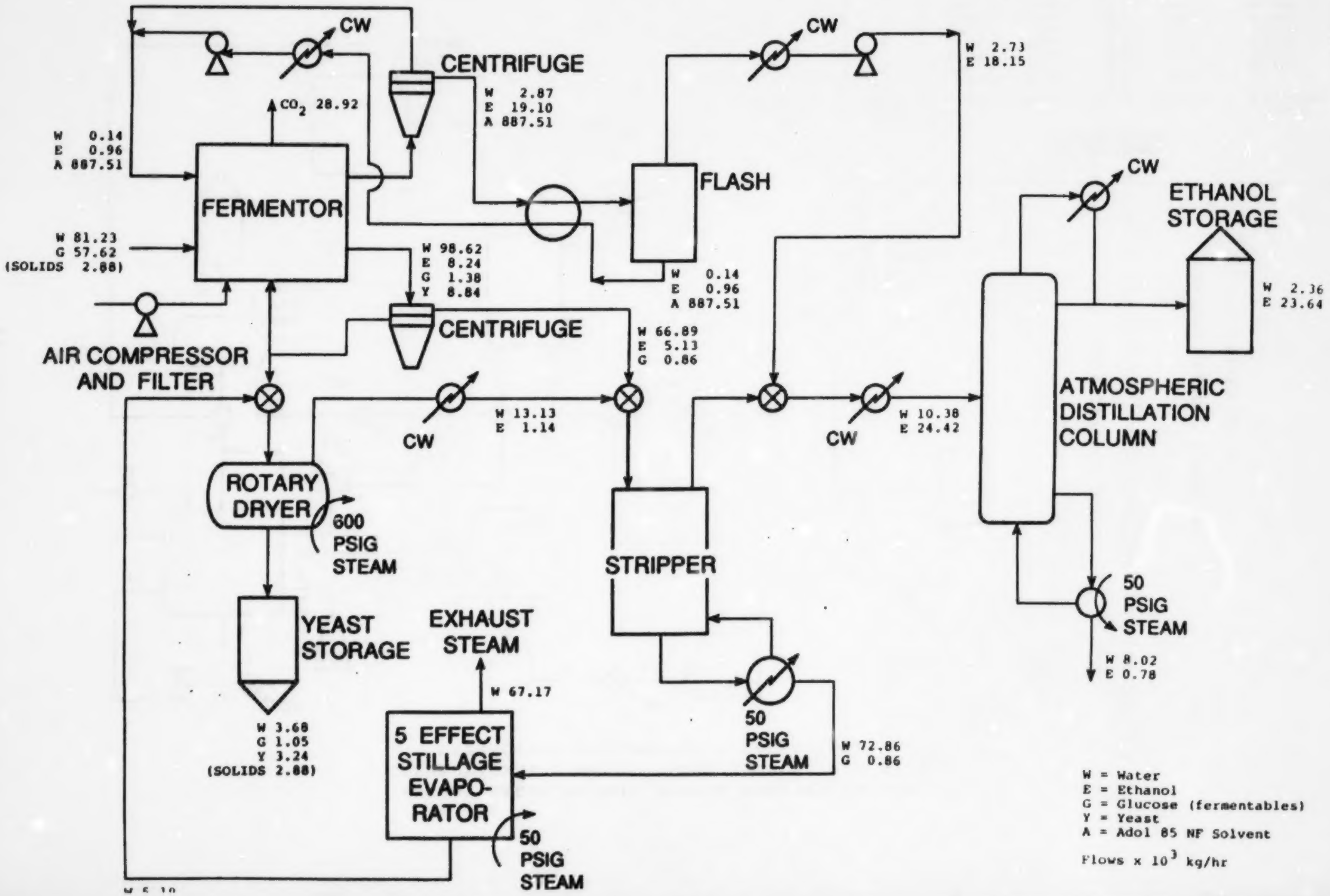
ENERGY BALANCES AND STREAM CONDITIONS FOR EXTRACTIVE FERMENTATION GRASS-ROOTS PLANT


1
2
4
10
CW
3
9
7
8
11
CW
12
13
5
6
19
21
CW
22
14
15
23
CW
24
CW
25
600
PSIG
STEAM
20
50
PSIG
STEAM
16
18
50
PSIG
STEAM
17
50
PSIG
STEAM
26

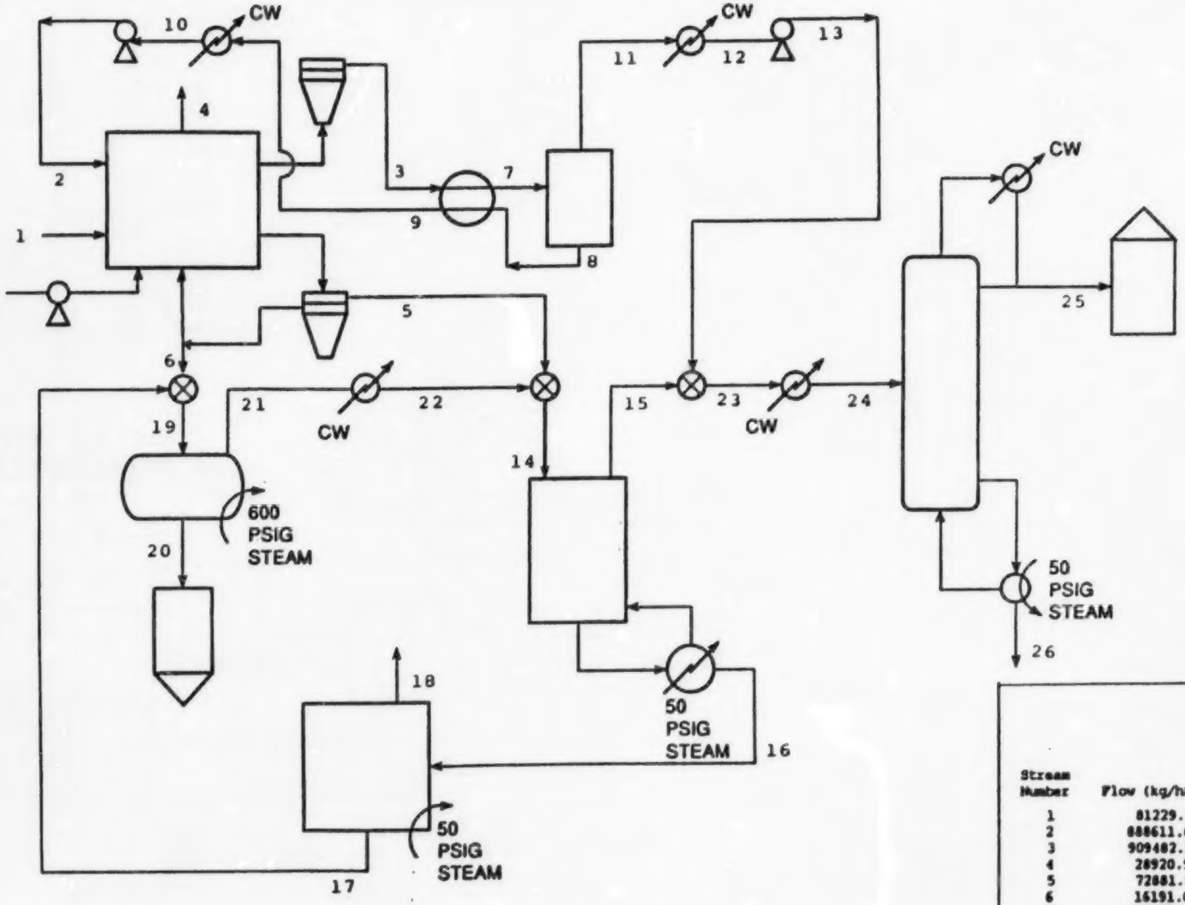
STREAM CONDITIONS
SIMULATION EF15

Stream Number	Flow (kg/hr)	Phase	T (°C)	P (psia)	Enthalpy (million kJ/hr)
1	31050.4	Liquid	30.0	14.7	
2	308569.5	Liquid	30.0	14.7	6.8727
3	316390.2	Liquid	30.0	14.7	9.1889
4	11128.5	Vapour	30.0	14.7	3.4346
5	27902.5	Liquid	30.0	14.7	0.8486
6	6230.2	Liquid	30.0	14.7	0.2007
7	316390.2	Liquid	90.6	14.7	56.0876
8	308569.3	Liquid	120.0	14.7	75.7494
9	308569.5	Liquid	60.0	14.7	28.8508
10	308569.5	Liquid	30.0	14.7	6.8730
11	7821.0	Vapour	120.0	14.7	10.4565
12	7821.0	Liquid	74.6	14.7	1.7918
13	7821.0	Liquid	74.6	14.7	1.7918
14	33606.4	Liquid	43.5	15.5	2.9121
15	5766.2	Vapour	94.2	15.3	11.2659
16	27840.2	Liquid	105.9	18.1	9.0189
17	2309.0	Liquid	106.7	18.1	0.6923
18	25532.0	Vapour	106.7	18.1	68.5868
19	8539.2	Mixed	44.7	25.0	0.8930
20	2835.2	Liquid	114.5	25.0	0.4973
21	5704.0	Vapour	114.5	25.0	14.6501
22	5704.0	Liquid	107.3	25.0	2.0636
23	13587.2	Mixed	95.3	25.0	13.0577
24	13587.2	Liquid	94.2	25.0	4.8649
25	10012.3	Liquid	78.8	15.0	2.3244
26	3576.[illegible]	Liquid	104.1	22.[illegible]	1.2399

MASS BALANCES FOR CONVENTIONAL PLANT RETROFITTED WITH EXTRACTIVE FERMENTATION TECHNOLOGY


CW
CENTRIFUGE
W 2.87
E 19.10
A 887.51
CO_2 28.92
W 0.14
E 0.96
A 887.51
FERMENTOR
W 81.23
G 57.62
(SOLIDS 2.88)
AIR COMPRESSOR AND FILTER
W 98.62
E 8.24
G 1.38
Y 8.84
CENTRIFUGE
FLASH
W 0.14
E 0.96
A 887.51
CW
W 2.73
E 18.15
W 66.89
E 5.13
G 0.86
CW
W 13.13
E 1.14
CW
W 10.38
E 24.42
ROTARY DRYER
600 PSIG STEAM
STRIPPER
50 PSIG STEAM
W 72.86
G 0.86
YEAST STORAGE
W 3.68
G 1.05
Y 3.24
(SOLIDS 2.88)
EXHAUST STEAM
W 67.17
5 EFFECT STILLAGE EVAPORATOR
50 PSIG STEAM
CW
ETHANOL STORAGE
W 2.36
E 23.64
ATMOSPHERIC DISTILLATION COLUMN
50 PSIG STEAM
W 8.02
E 0.78
W = Water
E = Ethanol
G = Glucose (fermentables)
Y = Yeast
A = Adol 85 NF Solvent
Flows x 10^3 kg/hr

ENERGY BALANCES AND STREAM CONDITIONS FOR CONVENTIONAL ETHANOL PLANT RETROFITTED WITH EXTRACTIVE FERMENTATION TECHNOLOGY


10
CW
4
2
1
3
7
9
8
11
CW
12
13
5
6
19
21
CW
22
14
600
PSIG
STEAM
20
15
23
CW
24
25
CW
50
PSIG
STEAM
26
50
PSIG
STEAM
16
18
50
PSIG
STEAM
17

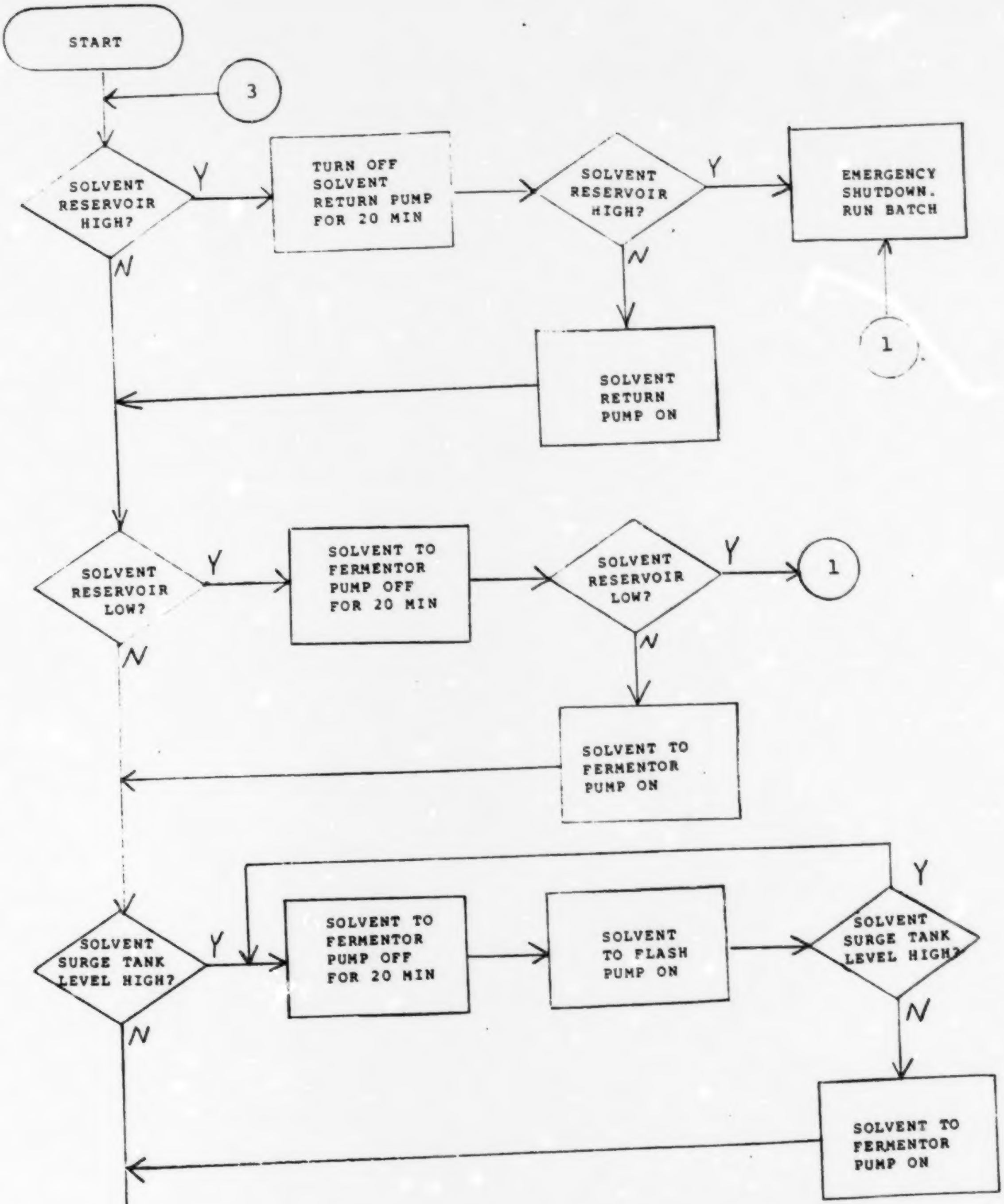
STREAM CONDITIONS
SIMULATION RETRONEW

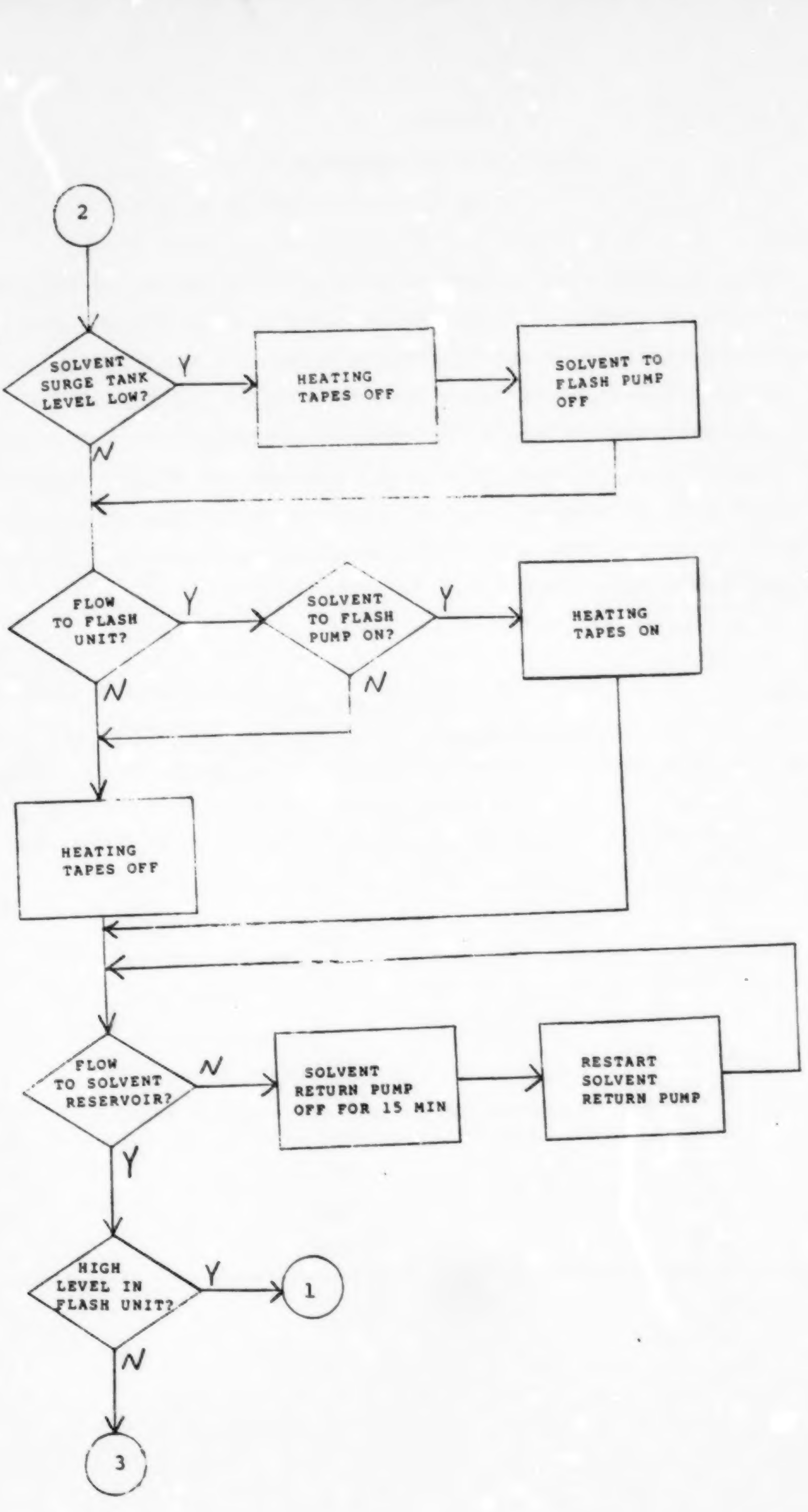
Stream Number	Flow (kg/hr)	Phase	T (°C)	P (psia)	Enthalpy (million kJ/hr)
1	81229.2	Liquid	30.0	14.7	
2	888611.6	Liquid	30.0	14.7	19.7676
3	909482.1	Liquid	30.0	14.7	25.9557
4	28920.9	Vapour	30.0	14.7	8.9258
5	72881.5	Liquid	30.0	14.7	2.0435
6	16191.0	Liquid	30.0	14.7	0.4849
7	909482.1	Liquid	90.7	14.7	161.0094
8	888611.1	Liquid	120.0	14.7	218.1117
9	888611.1	Liquid	60.0	14.7	83.0577
10	888611.6	Liquid	30.0	14.7	19.7684
11	20871.0	Vapour	120.0	14.7	27.9040
12	20871.0	Liquid	74.6	14.7	4.7817
13	20871.0	Liquid	74.6	14.7	4.7817
14	87145.6	Liquid	43.1	15.5	7.2040
15	13930.8	Vapour	94.2	15.3	27.2175
16	73214.9	Liquid	105.9	18.1	23.6194
17	6046.8	Liquid	106.7	18.1	1.7126
18	67167.3	Vapour	106.7	18.1	180.4322
19	22237.7	Mixed	44.6	25.0	2.1975
20	7973.5	Liquid	114.6	25.0	1.3767
21	14264.2	Vapour	114.6	25.0	36.7082
22	14264.2	Liquid	107.5	25.0	5.1605
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
24	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

APPENDIX C

CIM-PAC CONTROL ALGORITHM

This section presents the CIM-PAC™ algorithm used for controlling the Extractive Fermentation Process Demonstration Unit.


START
3
SOLVENT RESERVOIR HIGH?
Y
N
TURN OFF SOLVENT RETURN PUMP FOR 20 MIN
SOLVENT RESERVOIR HIGH?
Y
N
EMERGENCY SHUTDOWN. RUN BATCH
1
SOLVENT RETURN PUMP ON
SOLVENT RESERVOIR LOW?
Y
N
SOLVENT TO FERMENTOR PUMP OFF FOR 20 MIN
SOLVENT RESERVOIR LOW?
Y
N
1
SOLVENT TO FERMENTOR PUMP ON
SOLVENT SURGE TANK LEVEL HIGH?
Y
N
SOLVENT TO FERMENTOR PUMP OFF FOR 20 MIN
SOLVENT TO FLASH PUMP ON
SOLVENT SURGE TANK LEVEL HIGH?
Y
N
SOLVENT TO FERMENTOR PUMP ON
2


2
SOLVENT SURGE TANK LEVEL LOW?
Y
N
HEATING TAPES OFF
SOLVENT TO FLASH PUMP OFF
FLOW TO FLASH UNIT?
Y
N
SOLVENT TO FLASH PUMP ON?
Y
N
HEATING TAPES ON
HEATING TAPES OFF
FLOW TO SOLVENT RESERVOIR?
N
Y
SOLVENT RETURN PUMP OFF FOR 15 MIN
RESTART SOLVENT RETURN PUMP
HIGH LEVEL IN FLASH UNIT?
Y
N
1
3

APPENDIX D

FLASH CHARACTERIZATION TESTS

The flash unit was tested to determine the amount of ethanol removed under several different operating temperatures and pressures. The feed to the flash consisted of solvent with an ethanol concentration of 13.75 g/L. The solvent flowrate was 100 mL/min. The flash was warmed for four hours with the immersion heater before the tests were conducted. The air bleed valve was open allowing a small flow of air through the flash. The flashed solvent was continuously removed from the unit, which differs slightly from flash conditions during PDU operation in which the solvent is allowed a 15 minute holdup time before being removed.

The results of the tests are presented in Table D-1.

TABLE D-1

RESULTS FROM FLASH CHARACTERIZATION TESTS

Vacuum Pressure (inches Hg)	Flash Temperature (°C)	Ethanol Removed (%)
25.5	100	58
25.5	122	72
25.5	140	79
15.0	99	14
15.0	120	39
15.0	144	63
0.0	101	13
0.0	123	24
0.0	140	37
0.0	178	44

APPENDIX E

DATA FROM PDU RUNS

The Extractive Fermentation PDU was operated a total of nine times since the original commissioning. Since most of these runs consisted of equipment testing and PDU fine tuning, data for only the four runs discussed in this report are presented, and are shown in Tables E-2, E-3, E-4, and E-5, while a description of the main parameters can be found in Table E-1. Highlights of the other runs are presented, however, in Table E-6. Data for these other runs are available upon request.

TABLE E-1

MAIN PARAMETERS MONITORED DURING PDU OPERATION

T	Elapsed time (h)
P	Fermentor aqueous ethanol concentration (g/L)
X	Fermentor cell concentration (g dry wt/L)
S	Fermentor residual glucose concentration (g/L)
So	Glucose concentration in medium (g/L)
D	Aqueous dilution rate based on fermentor aqueous volume (h^{-1})
Ds	Solvent dilution rate based on fermentor aqueous volume (h^{-1})
Pu	Ethanol concentration in unflashed solvent (g/L)
Pf	Ethanol concentration in flashed solvent (g/L)
Vi	Cell viability (%)
Bu	Cell budding (%)
Vc	Volume of condensate collected (mL)
Pc	Ethanol concentration in condensate (% wt)

TABLE E-1

DATA FROM RUN # J198GSC

T	F	X	S	So	D	Ds	Pu	Pf	Vl	Bu	Vc	Pc
-1.0	44	4	47	179	0.00	0.00			89	22		
4.0	53	4	50	179	0.08	0.00						
8.0	51	7	45	179	0.08	0.00						
20.0	65	17	20	178	0.08	0.00			90			
24.0	66	18	19	178	0.10	0.00						
28.0	61	23	28	178	0.16	0.00						
34.0	70	32	2	193	0.16	0.00						
45.0	69	43	5	190	0.18	0.00			89	26		
48.5	70	43	4	190	0.20	0.00						
54.5	64	45	25	190	0.23	0.00						
57.5	65	45	23		0.23	0.00						
69.0	75	65	0		0.23	0.00			93	56		
72.5	80	65	14	198	0.23	0.00						
76.0	71	70	6	198	0.23	0.00						
80.0	69	69	15	198	0.23	0.00						
83.5	69	78	15	198	0.23	0.00						
92.0	65	79	10	187	0.23	0.00						
95.0	75	65	9	194	0.23	0.00			85	24		
96.0	64	65	7	194	0.23	0.00						
97.5	72	65	23	493	0.15	1.25						
100.5	62	65	60	493	0.15	1.25						
104.0	65	73	69	493	0.15	1.25	14.0	2.0				
107.5	74	61	73	493	0.15	1.25	14.0	2.0				
114.0	54	56	124	464	0.15	1.25	13.0	2.0			1700	47
118.0	60	63	95	464	0.14	1.25						
121.0	53	53	143	446	0.15	1.25	13.0	2.0	81	28		
125.0	58	61	147	446	0.15	1.25	13.5	1.5				
129.0	60	49	159	446	0.15	1.25	12.5	1.5			1745	49
131.5	58	87	134	472	0.15	1.25						
138.5	48	66	113	472	0.15	1.25					1400	48
143.0	49	65	209	522	0.15	1.25			76	34		
149.0	73	56	52	522	0.00	0.00						
153.0	61	55	64	499	0.12	1.25			74	18		
156.0	60	61	87	499	0.12	1.25					1580	45
162.0	60	62	83	479	0.12	1.25			75	33	900	43
166.0	56	60	61	479	0.12	1.25						
170.5	58	65	58	479	0.12	1.25	15.0	1.0				
175.0	61	63	46	479	0.12	1.25	13.5	1.5			1495	37
178.0	61	65	44	479	0.12	1.25	14.0	2.0				
182.0	61	63	34	495	0.12	1.25	13.5	2.0				
186.5	62	68	19	495	0.12	1.25	15.0	2.0			1200	30
191.0	66	72	29	520	0.12	1.25			75	37		
195.0	65	69	44	520	0.12	1.25	14.0	2.5				
198.0	63	72	53	520	0.12	1.25	15.0	2.0			1800	51

TABLE E-2

DATA FROM RUN # AU90MOSC

T	P	X	S	So	D	Ds	Pu	Pf	Vi	Bu	Vc	Pc
0.0	52	40	34	156	0.09	0.00						
3.0	54	96	35	156	0.10	0.00						
8.0	48	70	42	156	0.10	0.00			75	6		
20.0	42	44	56	156	0.10	0.00						
24.0	46	63	54	156	0.10	0.00						
26.5	51	66	42	156	0.10	0.00						
28.0	48	70	39	156	0.10	0.98						
31.0	39	70	22	156	0.10	0.98						
35.0	33	70	18	156	0.10	0.98	6.8	2.8				
45.0	23	68	18	156	0.10	0.98			80	12	650	34
48.0	25	75	20	156	0.10	0.98	6.0	2.9				
51.0	25	70	16	269	0.10	0.98	5.5	2.8				
54.0	29	70	28	269	0.10	0.98	7.3	3.4				
58.0	33	76	33	269	0.10	0.98	7.3	3.0				
68.0	32	73	40	269	0.10	0.98					1100	36
72.0	28	58	56	269	0.10	0.98	6.4	2.6				
76.0	28	64	53	269	0.10	0.98	7.3	3.1	74	11		
80.0	28	60	47	269	0.10	0.98	7.4	3.3				
82.5	26		67	371	0.10	0.98						
90.0	12	45	148	371	0.10	0.98						
94.0	26			371	0.10	0.98						
97.0	25	48	86	371	0.10	0.98						
100.0	37	48	57	371	0.10	0.98						
103.0	25	52	99	371	0.10	0.98	7.1	3.7				
106.0	24	40	108	371	0.10	0.98						

Note: Due to unreliable viability tests, the reported cell concentration in Table E-2 is the measured cell concentration, not the live cell concentration.

TABLE E-3

DATA FROM RUN # SE90HYSC

T	P	X	S	So	D	Ds	Pu	Pf	Vi	Bu	Vc	Pc
0.0	60	50	18	175	0.11	0.00						
4.0	58	44	15	175	0.16	0.00						
9.0	52	44	21	175	0.16	0.00						
21.5	53	44	7	175	0.16	0.00						
23.0	54	44	3	175	0.20	0.00			95	8		
26.0	62	46	32	175	0.15	1.25	12.6	2.3				
31.0	54	50	36	335	0.15	1.25	12.7	2.2	89	17		
35.0	56	52	19	335	0.15	1.25	11.4	1.7				
41.5	47	44	15	335	0.15	1.25						
44.5	51	55	13	335	0.15	1.25					2000	36
48.0				335	0.15	1.25	10.0	1.8	92	35		
48.5	53	50	14	335	0.15	1.25	10.7	1.7				
53.0	55	56	26	335	0.18	1.25	10.9	1.7				
58.0	51	48	29	335	0.18	1.25						
66.5	44	37	50	335	0.18	1.25	9.2	1.4			1950	26
72.0	41	65	28	335	0.18	1.25						
80.0	37	30	78	335	0.18	1.48			91	66		
83.0	43	30	84	335	0.18	1.48						
90.0	42	34	57	335	0.18	1.48	10.2	1.8			1550	20
94.0	45	34	72	335	0.18	1.48			89	49		
96.0	47	35	70	335	0.18	1.48						
101.0	40	35	91	335	0.18	1.48			91	52		
105.0	40	33	131	335	0.18	1.48						
116.5	31	44	140	335	0.18	1.48					2500	43
119.0	37	46	169	335	0.18	1.48			91	15		
122.0				335	0.18	1.48					400	25

TABLE E-4

DATA FROM RUN # AG90GLZM

T	P	X	S	So	D	Ds	Pu	Pf	Vc	Pc
0.0	67	3.0	3	200	0.11	0.00				
3.0	60	1.8	47	200	0.11	0.00				
7.0	40	1.3	93	200	0.11	0.00				
19.0	43	2.1	51	200	0.11	0.00				
23.0	51	2.0	46	200	0.11	0.00				
26.0	45	1.8	55	200	0.11	0.00				
27.0	47	1.9	57	200	0.12	0.00				
29.0	43	1.4	62	190	0.12	1.25	6.8	2.1		
32.5	35	2.0	93	440	0.08	1.25				
34.0	43	2.3	101	440	0.08	1.25	7.4	2.9		
42.5	95	3.8	0	440	0.08	1.25				
44.0				440	0.08	1.25			700	32
46.0	72	3.0	47	440	0.08	1.25	10.0	4.2		
49.0	55	2.5	76	440	0.08	1.25			550	50
52.0	49	2.8	105	440	0.08	1.25	8.3	3.2		
55.0	52	2.8	81	440	0.08	1.25				
58.5	56	3.5	48	440	0.08	1.25				
65.0	58	4.3	58	472	0.08	1.25	10.2	3.3	1600	44
69.0	63	3.4	47	472	0.08	1.25				
73.0	58	3.0	66	472	0.08	1.25				
76.0	48	3.1	82	472	0.08	1.25				
80.0	48	3.0	77	477	0.08	1.25	8.8	2.9		
81.0				477	0.08	1.25			1650	58
89.0	38	1.8	160	477	0.08	1.25			550	43
93.5	30	1.6	202	477	0.08	1.25	5.1	2.9	250	42
97.5	36	1.4	199	477	0.08	1.25				

TABLE E-6

HIGHLIGHTS FROM PREVIOUS PDU RUNS

Major Runs	Operating Time (days)	Purpose/Result
#J240GSC	10	PDU commissioning Demonstrated long-term stable operation of system Tested process control system
#N647GSC	27	Investigated effect of salts in medium Tested condensor cooling system Tested flash unit spray nozzle Test for fermentation by-products Studied effect of aeration
#A504GSC	20	Developed operating manual Cell concentration control Achieved 67 wt% ethanol in condensate
#M290GSC	12	Tested fermentor aqueous level control system Achieved predicted optimum conditions

APPENDIX F

FURTHER EXTRACTIVE FERMENTATION DATA

Since this report was first drafted, several more runs with the Extractive Fermentation Process Demonstration Unit have been performed. To address earlier concerns about long-term steady state operation and the performance of *Zymomonas mobilis* under Extractive Fermentation conditions, data from two runs - one using yeast and the other *Zymomonas mobilis* - are presented. Only the highlights from these runs are shown. Detailed descriptions are available upon request.

RUN #FEB0991/*Saccharamyces cerevisiae*

The Extractive Fermentation Process Demonstration Unit was operated between February 11 and February 19, 1991 for a total of 184.5 hours. The purpose of the run was to obtain steady state Extractive Fermentation data for a medium concentration feed (≈ 400 g/L glucose) and a high concentration feed (≈ 500 g/L glucose) and compare it to the optimum for conventional fermentation. The fermenting organism was a strain of *Saccharamyces cerevisiae*.

One steady state for conventional fermentation was obtained, at a feed concentration of 195 g/L glucose and an aqueous dilution rate of 0.23 hr^{-1}. The fermentor conditions were 71 g/L ethanol, 44 g/L live cells and 14 g/L residual glucose, while ethanol productivity was 15.7 g/L·hr. Ethanol and cell yields were 0.399 g/g and 0.0226 g/g, respectively. This steady state was maintained for approximately 21 hours.

A steady state was obtained during Extractive Fermentation with a feed concentration of 413 g/L glucose and aqueous and solvent dilution rates at 0.14 hr^{-1} and 1.55 hr^{-1} respectively. Steady state fermentor conditions were 60 g/L ethanol, 53 g/L live cells and 36 g/L residual glucose, respectively. Ethanol productivity was 26.2 g/L·hr, while ethanol and cell yields were 0.369 g/g and 0.0336 g/g, respectively. Fermentor conditions were at steady state for approximately 14 hours.

A steady state was also obtained during Extractive Fermentation with an average feed concentration of 495 g/L glucose. Steady state fermentor conditions were 67 g/L ethanol, 59 g/L live cells and 26 g/L residual glucose, respectively. Ethanol productivity was at 32.7 g/L·hr. Ethanol and cell yields were 0.402 g/g and 0.0131 g/g, respectively. Steady state conditions were maintained for about 36.5 hours.

Fermentor conditions and ethanol productivity are presented in Figure F-1 and F-2, respectively.

FIGURE F-1

FERMENTOR CONDITIONS
During Run # FEB0991/SC

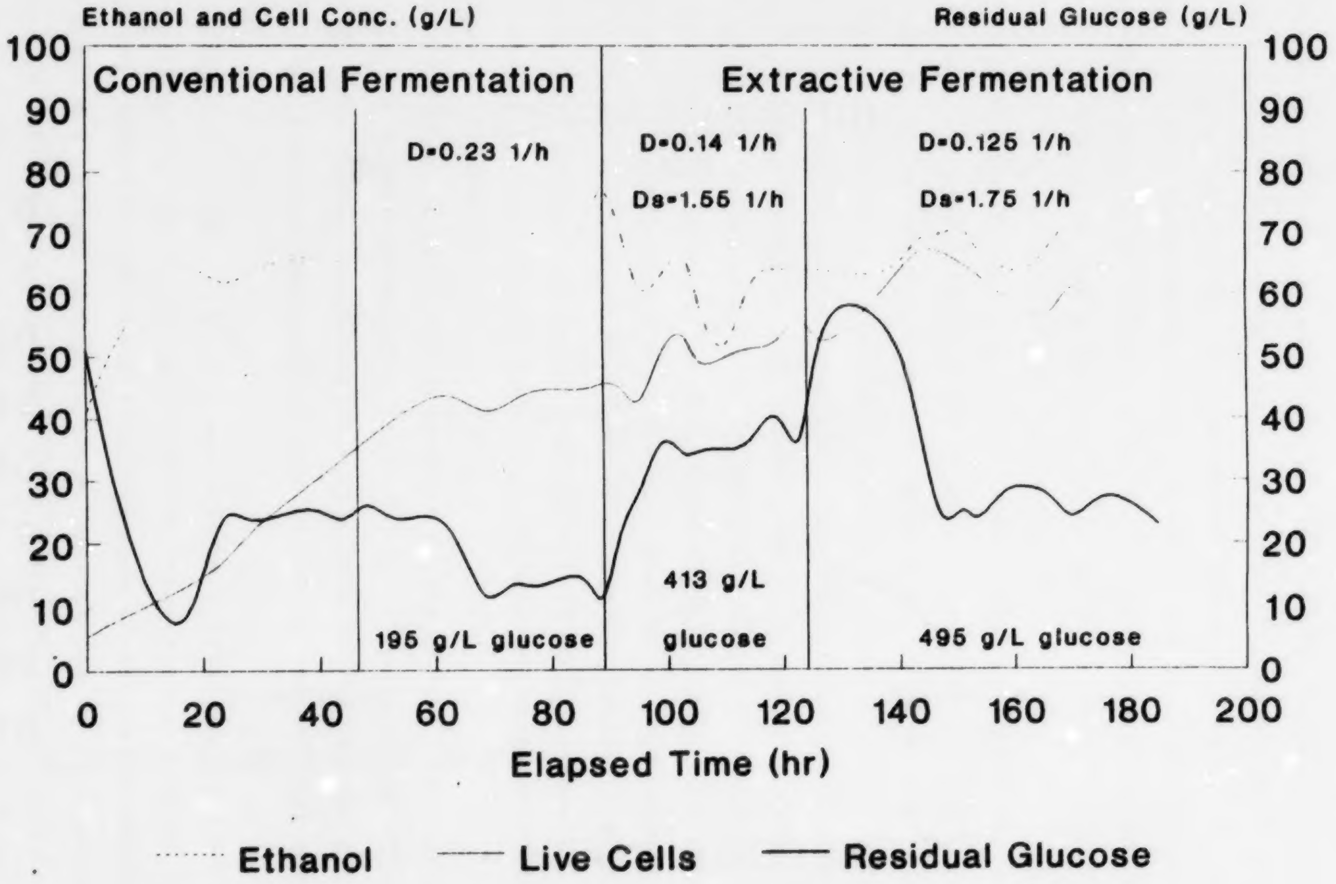
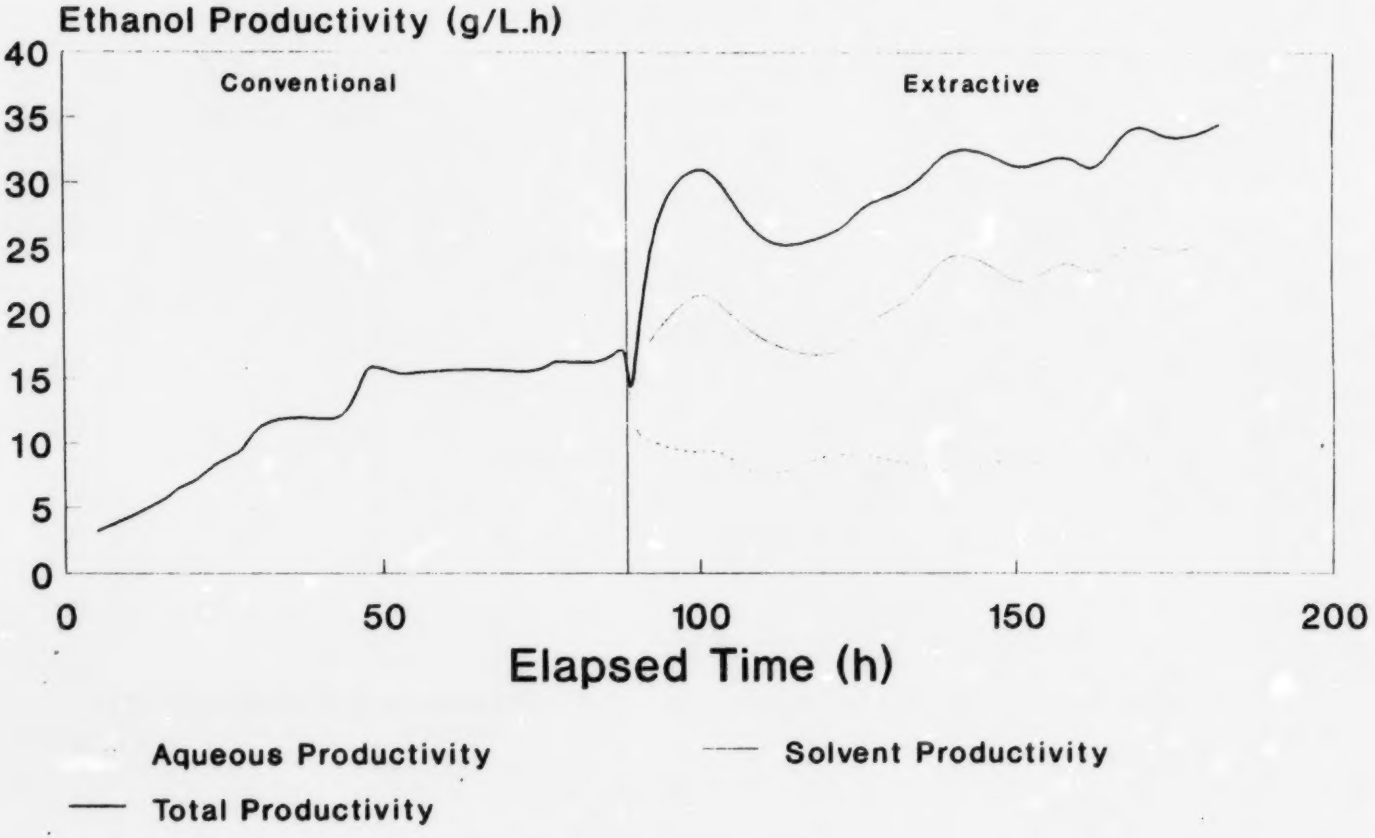


FIGURE F-2

ETHANOL PRODUCTIVITY

From Run # FEB0991/SC



RUN # JAN1091/*Zymomonas mobilis*

The purpose of run # JAN1091/ZM was to observe the performance of *Zymomonas mobilis* under steady state Extractive Fermentation conditions using a synthetic glucose medium. The system was operated a total of 224 hours. During this time, three steady state periods - one of conventional fermentation, and two of Extractive Fermentation - were achieved (steady state being defined as the time after which 14.4 L - three reactor volumes - of medium had passed through the fermentor).

During the first twenty hours of conventional operation the medium flowrate was adjusted between 8 and 11 mL/min, and finally set to 9 mL/min, resulting in an aqueous dilution rate of 0.11 h^{-1}. The average feed concentration was 134 g/L glucose. This low concentration was purposely chosen to prevent the ethanol concentration from reaching inhibitory levels; however, as shown in Figure F-3, ethanol was high enough to cause sinusoidal "cycling" which is characteristic of *Zymomonas mobilis*. Although ethanol and residual glucose concentrations fluctuated significantly, cell concentration was relatively stable over the entire period at an average concentration of 1.6 g/L. After three reactor volumes of medium had passed through the fermentor, the average fermentor conditions were 40 g/L ethanol, 1.6 g/L cells, and 34 g/L residual glucose, respectively. Fermentor conditions are presented in Figure F-3.

At 73.0 hours solvent was introduced to the fermentor at a flowrate of 92 mL/min, or a solvent dilution rate of 1.15 h^{-1} based on an aqueous volume of 4.8 L. The medium concentration during this period was 280 g/L glucose, and the feed flowrate remained the same as during conventional operation (9 mL/min).

As shown in Figure F-3 there was a large initial increase in residual glucose following the switch to Extractive Fermentation; however, no flow adjustments were made and the residual glucose concentration decreased to a minimum value of 23 g/L within 16 hours, accompanied by a corresponding increase in ethanol concentration. Over time, the ethanol cycling appeared to have been dampened, compared to the conventional period. Cell concentration tended to cycle somewhat, with maximum values coinciding with decreasing residual glucose concentrations. The average cell concentration over the "steady state" period was 3.8 g/L, while the range was 2.3 - 4.9

g/L. Mean values for ethanol and residual glucose, after three reactor volumes of medium had passed through the fermentor, were 47 g/L and 58 g/L, respectively.

A second Extractive Fermentation period was initiated by increasing the solvent flowrate to the fermentor. The solvent flowrate was increased to 120 mL/min, or a dilution rate of 1.5 h^{-1} at a fermentor aqueous volume of 4.8 L. The feed flowrate remained unchanged at 9 mL/min, and the average feed concentration was 295 g/L glucose.

As shown in Figure F-3, there appeared to be a brief initial cycling period, after which the residual glucose began a steady decline, while ethanol and cell concentrations slowly increased. In contrast to the first Extractive Fermentation period, a steady state with unchanging conditions was achieved during the last nine hours of the run. Ethanol concentration remained steady at 55 g/L, while cell concentration ranged from 5.8 - 6.0 g/L. Residual glucose was between 0 - 7 g/L. Average values for the last nine hours were 55 g/L ethanol, 5.9 g/L cells, and 4 g/L residual glucose. Ethanol productivity for all periods is presented in Figure F-4.

This run is significant in that it illustrates how Extractive Fermentation can be used to dampen and eventually eliminate the cycling behaviour of *Zymomonas mobilis* and thus eliminate the associated downstream difficulties with process control.

FERMENTOR CONDITIONS

During Run # JAN1091/ZM

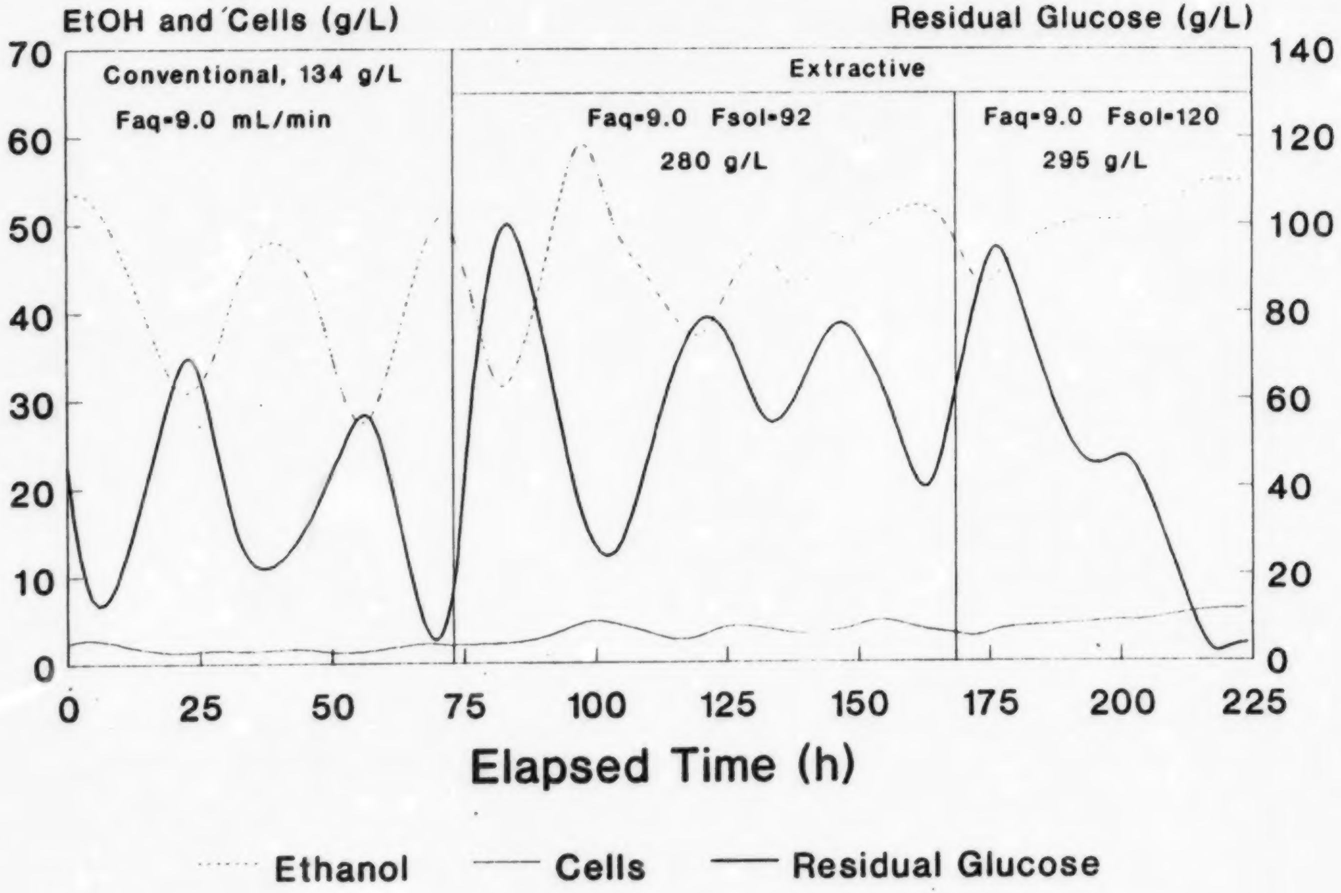
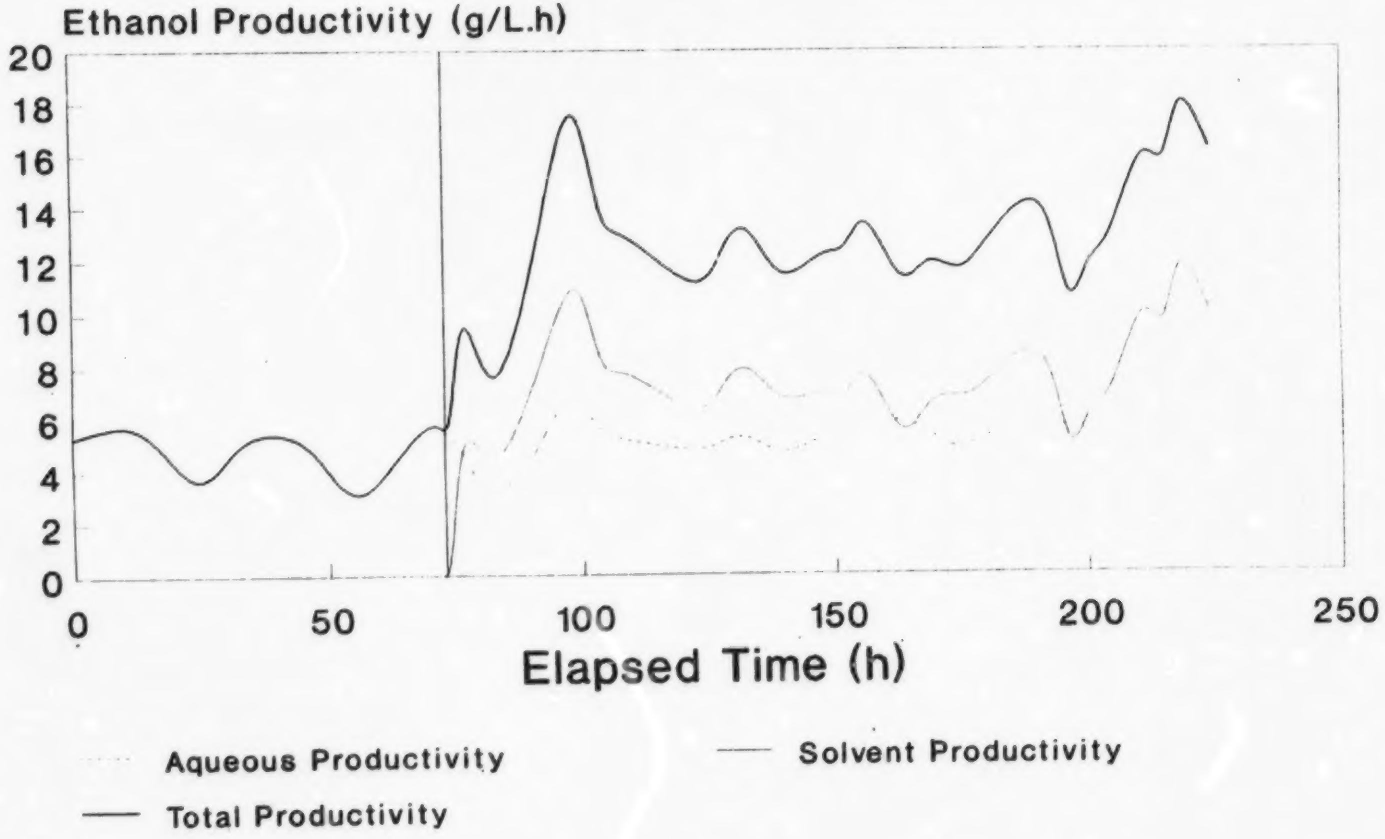


FIGURE F-4

ETHANOL PRODUCTIVITY

From Run # JAN1091/ZM



APPENDIX G

EXTRACTIVE FERMENTATION BUSINESS PLAN

This section details the business plan developed for commercialization of Extractive Fermentation technology.

BUSINESS PLAN

CONTINUOUS EXTRACTIVE FERMENTATION
FOR
ETHANOL PRODUCTION

October 1990

EXECUTIVE SUMMARY

A novel extractive fermentation technology for the production of ethanol has been developed at Queen's University at Kingston, Ontario. This technology is more economical than conventional fermentation technologies and will allow producers to improve efficiency and profitability. PARTEQ Research and Development Innovations (PARTEQ), the technology transfer arm of Queen's University has the exclusive rights to this technology and is currently seeking licensees/or joint venture partners.

Ethanol can be made from ethylene or by the fermentation of sugars. Production of ethanol by fermentation is hampered by a phenomenon known as end-product inhibition. That is, the condition where high ethanol concentrations in the fermentation broth decrease the actual ethanol production rate. In 1982 a research group, under the direction of Dr. Andrew Daugulis, began investigating the feasibility of continuous extraction of ethanol from the broths through the use of a water-immiscible organic solvent. A solvent screening program was undertaken to assess over 1400 solvents and a suitable solvent, comprised mainly of oleyl alcohol, was eventually selected. An operational prototype system using a 1.3-litre continuous stirred tank fermentor was then constructed and the system was upgraded in 1986 to include a 7-litre continuous stirred tank fermentor and a flash vaporization unit. This system was further upgraded in 1988 to incorporate computerized process monitoring and control and to add more sophisticated equipment to allow cell-recycle and solvent recovery.

In our process, a sugar feed is fermented in an aqueous broth containing salts and a yeast extract (Saccharomyces cerevisiae). The solvent, which has a high affinity for ethanol and a high partition co-efficient to permit inexpensive separation, is introduced to the fermentor. Due to the difference in the densities between the solvent and the water, the solvent rises to form a second phase at the top of the fermentor. The solvent is non-toxic to the microorganism and remains stable. Although the fermentation broth is continuously stirred to provide adequate mixing, a relatively stagnant solvent layer remains in the upper part of the fermentor. An overflow pump feeds primarily solvent input into a three-way centrifuge. Water and cells in that stream are returned back to the fermentor and the ethanol-laden solvent is fed into a vacuum flash vaporization unit. Essentially, all of the ethanol is vaporized and then condensed in a distillation column. The solvent is returned to the solvent reservoir for reuse. Ethanol recovery from the solvent stream is as high as 70% by weight without distillation.

We feel that our technology is well protected. The technology is covered by U.S. Patent #4,865,973 which issued September 12, 1989, to which we will have an exclusive license. We assess the protection as broad and strong and note that it also covers the process for other applications in addition to ethanol production. Patent applications have been filed in Canada, Japan, Britain, Germany, Sweden and France.

Extensive computer simulation has been done in order to evaluate the economic advantages of our ethanol production process relative to conventional fermentation technology. Our process allows a savings ranging from 6-16¢ (CDN) per litre depending on whether the technology is used in a new plant or incorporated as a retrofit. This savings represents considerable additional profit potential for ethanol producers using our process. Although our process may have potential for the production of industrial use ethanol, our focus will be primarily on the use of ethanol as a fuel or in combination with conventional fuels. Concern about energy fuel supplies in the

1970s created a major emphasis on developing alternative fuels and the focus on alternative fuels will undoubtedly receive renewed emphasis resulting from the recent events in the Persian Gulf surrounding Iraq's invasion of Kuwait. The concerns related to energy dependence have been supplemented lately by environmental concerns over the use of lead and carbon monoxide emissions. Oxygenates (gasolines which are low percentage blends of alcohols and ethers) in gasoline, represent only approximately 3% of the U.S. production of 300 million gallons per day. It has been projected that potential oxygenate demand could grow to 3.5 billion U.S. gallons by 1995 and ethanol represents about 1/3 of the total oxygenate supply. Increased demand for fuel ethanol in the U.S. will however depend greatly on whether the price for oil remains at its currently elevated level and whether the U.S. Federal tax credit gets extended beyond 1992. Producers are awaiting assurance that such a credit will continue before expansion proceeds. All indications are that this legislation will pass within the next 6 months and the demand for ethanol will realize significant growth.

Commercialization could take many forms from straight exclusive licensing through to the building and operation of an ethanol plant. Initial concentration was on attracting one or more licensees. More recent efforts have been focussed on building a joint venture or a new, entrepreneurial venture to support scale-up and commercialize the technology. It is clear that a pilot plant and economic and technical information from such a facility is required in order to successfully commercialize this technology. PARTEQ is continuing with its venture and license efforts while also attempting to attract funds from various sources for pilot plant development. What is clear is that we have ethanol production technology which will provide producers with significant economic advantage. When the ethanol industry gets revived, as we are confident it will, in the very near term, our technology will be in high demand.

GENERAL

Ethanol was used as a fuel as far back as the 1800s. Ethanol was in fact the fuel on which the Model T was designed to run. It was only after World War II, when petroleum based gasoline became readily available, that alcohol fuels were deemphasized. However, the oil crisis of the 1970s and the current environmental concerns have prompted a resurgence in fuel alcohols.

Ethanol can be made from ethylene or by the fermentation of sugars. Production of ethanol by fermentation is hampered by a phenomenon known as end-product inhibition. That is, the condition where high ethanol concentrations in the fermentation broth decrease the actual ethanol production rate.

In 1982, a research group, under the direction of Dr. Andrew Daugulis, at Queen's University in Kingston, Ontario began investigating the feasibility of continuous extraction of ethanol from the broth through the use of a water-immiscible organic solvent. A solvent screening program was undertaken to assess over 1400 solvents and, based on theoretical screening of predicted physical and chemical properties, 70 solvents were subsequently investigated experimentally.

A solvent comprised mainly of oleyl alcohol was selected as the solvent with the most preferred characteristics. An operational prototype system using a 1.3-litre continuous stirred tank fermentor was then constructed and results indicated a near complete conversion of a concentrated glucose feed.

In 1986 the system was upgraded to include a 7-litre continuous stirred tank fermentor and a flash vaporization unit to thermally recover ethanol from the solvent stream. Continuous, long-term operation of the system again showed excellent ethanol recovery with a relatively concentrated glucose feed.

The system was further upgraded in 1988 to incorporate computerized process monitoring and control and to add more sophisticated equipment to allow cell recycle and solvent recovery. An economic simulation was then done to assess the relative competitiveness of conventional fermentation of ethanol compared to the solvent extractive fermentation process.

PARTEQ Research and Development Innovations (PARTEQ) has the exclusive worldwide royalty free rights to this technology and has presented the technology to ethanol producers and ethanol plant design engineering firms in an attempt to license the technology. Although there is significant interest in the technology, producers and design firms are reluctant to acquire technology until tax incentive legislation, currently in place in the U.S. until 1992, is extended for the rest of this decade. The ethanol industry has had to rely on tax incentives and has been slow to modernize. The recent push towards alternative fuels and clean air legislation will create a heavy demand for ethanol in this next decade and beyond. Indications are that the tax incentive in the U.S. will be extended and we wish to be well positioned to take advantage of the demand for new technology.

TECHNOLOGY

Our process provides a method for producing ethanol by extractive fermentation. A sugar feed is fermented on a continuous basis in an aqueous broth by the yeast Saccharomyces cerevisiae. A solvent comprised substantially of oleyl alcohol which has a high affinity for ethanol and a high partition co-efficient is continuously introduced to the fermentor. Due to the difference in the densities between the solvent and fermentation broth, the solvent rises to form a second phase at the top of the fermentor. The solvent is non-toxic to the microorganism and other than acting as an extractant for ethanol, is essentially inert. Although the fermentation broth is continuously stirred to provide adequate mixing, a relatively stagnant solvent layer remains in the upper part of the fermentor. An overflow pump feeds primarily solvent input into a three-way centrifuge. Water and cells in that stream are returned back to the fermentor and the ethanol-laden solvent is fed into a flash vaporization unit. Essentially, all of the ethanol is vaporized and then condensed. The solvent, being non-volatile, is returned to the solvent reservoir for reuse.

The system has been run on a continuous basis for up to 800 hours. No toxic or inhibitory compounds were evident and no deterioration of the physico-chemical properties of the solvent were observed. Ethanol recovery from the solvent stream was high (70% by weight ethanol, the remainder water).

A computer operated process control system using Cim-Pac software was incorporated into our process demonstration unit. This system allows individual on-off control of all pumps and continuous data logging of all levels, temperatures, pressures, etc. Real time display of all parameters superimposed over a computer generated process flow sheet is also available with our system.

Extensive computer simulation has been done in order to evaluate the economic advantages of our ethanol production process relative to conventional fermentation technology (continuous fermentation with cell recycle). Software was written to simulate the operation of a continuous stirred tank fermentor with cell recycle under conventional and extractive fermentation conditions. This program was incorporated into a commercially available simulation package, PROCESS^{TM}, so that both extractive and conventional fermentation could be modelled. Examination of the process flow sheets in Appendix 1 will show that efforts were made to keep our process as close as possible to the conventional approach. This was done so as to reduce complexity and enhance acceptance of our process for retrofit applications.

PROCESS ECONOMICS

The economic model simulated the operation of both processes over a wide range of operating conditions for a plant capacity of 100 million litres per year of azeotropic ethanol. Common assumptions involving equipment costs, utilities, labour, etc. were used with conventional chemical engineering financial analysis in order to provide a direct comparison of the two processes on the same basis. Assumptions were discussed with industry so as to confirm their applicability.

Appendix 2 contains a number of the assumptions used with respect to operating conditions, raw materials, utilities costs, operational costs and capital equipment and investment cost factors. Operating conditions for the extractive fermentation process were based on conditions experienced in our demonstration unit.

It is important to note that the absolute figures obtained for production costs depend on the costs of energy, raw materials, by-product prices, etc. which are in turn very dependent on market conditions. Our prices and costs are based on January 1989 Canadian dollars.

The simulation was performed to identify optimum economic operating conditions for both processes and then to compare production costs based on those operating conditions and on a wide range of variations of those conditions.

The optimum operating conditions in terms of concentration of fermentables and aqueous dilution rates are shown in Appendix 3 for the conventional fermentation process. The optimum condition of 18% fermentables and 0.2 hours^{-1} dilution rate (reciprocal of hydraulic retention time) was then used to determine a production cost of 45.1¢ per litre. A detailed cost breakdown for that simulation is provided in Appendix 4.

For the extractive process, the solvent dilution rate was also varied. The optimum operating conditions can be seen from the charts shown as Appendix 5. The optimum conditions proved to be an aqueous dilution rate of 0.15 hours^{-1}, a solvent dilution rate of 1.25 hours^{-1} and a feed concentration of 41.5% fermentables. These conditions resulted in a production cost of 38.7¢ per litre and a detailed cost breakdown for that simulation is provided in Appendix 6.

The extractive fermentation process is very suitable for retrofit situations. The technology was maintained as close to conventional as possible so that retrofit could be easily introduced. Simulations for retrofit scenarios were done by using the above described optimum operating conditions for the extractive fermentation case. The increased feed concentration and the significantly reduced water requirements result in a corresponding rise in plant capacity. For example, the production cost was reduced to 32.7¢ per litre (conventional price was 45.1¢ per litre) with a 94% increase in plant capacity.

It was found however that even with the above retrofit, capacity for downstream equipment was not fully utilized. A higher aqueous dilution rate of 0.218 hours^{-1} (previously 0.15 hours^{-1}) was used in order to make full use of existing plant equipment capacity. Plant capacity increased from 100 million litres per year to 255 million litres and the production cost dropped to 29.4¢ per litre. Capital investment, including solvent costs, for this retrofit was $6.8 million. A detailed cost breakdown for that simulation is provided as Appendix 7. Payback of capital investment is in the order of a few months.

For ease of presentation, the absolute figures obtained for optimum operating conditions are shown below:

	Production Cost
Conventional fermentation (new)	45.1 cents/L
Extractive fermentation (new)	38.7 cents/L
Extractive fermentation (retrofit)	29.4 cents/L

We stress that the simulation was done to determine the relative competitiveness of producing ethanol by our extractive fermentation process. A savings ranging from 6-16¢ per litre is very significant and represents considerable additional profit potential for ethanol producers using our process.

MARKET POTENTIAL

Ethanol is used in a number of ways. For industrial applications, ethanol is used for such things as tobacco flavouring, paints and paint supplements, liquid detergents, cosmetics, pharmaceuticals and thinning solvents. Ethanol is of course also used for consumption in the beverage alcohol industry. Although our process may have potential for industrial use ethanol, the trace of a solvent may present regulatory hurdles. We will therefore focus our analysis on the market for ethanol for use in fuels. As only approximately 80 million litres of ethanol are produced in Canada, we shall concentrate our analysis on the U.S. fuel ethanol market.

Ethanol can be used as a fuel either alone (neat) or in combination with conventional fuels. Oxygenated gasolines are low-percentage blends of alcohols and ethers in gasoline. Oxygenates displace part of the oil required to make gasoline and provide an added feature of acting as an octane enhancer. Ethanol is an oxygenate and is used in large volumes in gasohol, a blend of 90% unleaded gasoline and 10% ethanol. Ethanol can also be used alone as a fuel and although neat ethanol has seen extensive use in Brazil where 1/3 of the country's 10 million automobiles operate on neat ethanol, it is not likely that neat ethanol will be used extensively in North America, at least for some time. Use of neat ethanol would require significant engine modification.

Interest in alternative fuels was given emphasis in the 1970s because of concern about energy fuel supplies. Recently, alternative fuels have received renewed emphasis because of environmental concerns. The mandated phase down of lead additives in gasoline and the concern over the use of aromatics (benzene, toluene, xylene) as octane enhancers has served to increase the interest in oxygenated fuels. Oxygenates have become important in improving air quality. Carbon monoxide produced mainly from vehicles is a serious environmental concern and the use of oxygenates serves to reduce that problem. A number of States in the U.S. have in fact acted to encourage the use of oxygenates. Fuel supply is however still a major concern in the U.S. and it is recognized that reliance on oil imports will grow and that the most promising means to reduce oil consumption is through the use of alternative fuels. Recent events in the Persian Gulf surrounding Iraq's invasion of Kuwait have served to highlight the US dependence on oil imports. We now fully anticipate seeing once again a renewed emphasis on the importance of alternative fuels.

Gasoline is produced in the U.S. at the rate of 300 million gallons per day. Oxygenates represent only approximately 3% of that total. This however represents a significant growth in oxygenates from 0.8% in 1983. It has been projected that potential oxygenate demand could grow to 3.5 billion U.S. gallons by 1995. Ethanol represents about 1/3 of the total oxygenate supply. At present MTBE, methanol and ethanol have roughly an equal share of the oxygenate market.

Government subsidies through tax credits are an important consideration in the assessment of the market for fuel ethanol. Through Federal gasoline excise tax exemptions, companies receive the equivalent of 60¢ per gallon in subsidies. In addition, numerous States provide tax breaks ranging from 1¢ through to 16¢ per gallon for blended fuel. The average State exemption amounts to an additional 20¢ per gallon of ethanol. Appendix 8 shows the combined State and Federal tax exemptions along with the ranges of gasohol (10% ethanol) sales as a percent of total gasoline sales as of April 1989.

Increased demand for fuel ethanol in the U.S. will depend greatly on whether the price of oil remains at its currently elevated level and whether the U.S. Federal tax credit gets extended beyond 1992. Producers are awaiting for assurance that such a credit will continue before expansion proceeds. President Bush is on record as supporting the extension and the conjecture is that the extension will be approved. Ethanol has the advantage of being derived from agricultural products such as corn, wheat, barley, sugar beets and sugar cane and the U.S. government has a strong interest in maintaining the markets for those products. Although methanol and other oxygenates (notably MTBE) will be popular and less expensive, subsidies for ethanol producers will likely insure ethanol's competitiveness. A technology to significantly reduce the cost of ethanol production will undoubtedly be very popular.

There are approximately 65 fuel ethanol plants now operating in the U.S. with the majority employing fermentation, either corn wet or dry milling facilities. A list of producers as of January 1989 is shown in Appendix 9. It is important to note that although we anticipate our process can be used with dry milling operations, i.e. solids can be processed, we have thus far only demonstrated the system with liquid feed. It is therefore most applicable to wet milling, molasses and sugar cane based plants.

Archer-Daniels-Midland Company from Decatur, Illinois operates 3 large wet milling plants and enjoys approximately 50% of the U.S. ethanol market. Other significant players in the wet mill category are listed below:

Grain Processing Corporation - Muscatine, Iowa
AE Staley - Rolling Meadows, Illinois
Pekin Energy - Pekin, Illinois
Minnesota Corn Processors - Marshall, Minnesota

A medium sized wet mill plant (12 million gallons per year) will be in place for Hubinger Company of Keokuk, Iowa but it is not yet operating. Also a large 35 million gallon per year molasses based plant is in place in New Iberia, Louisiana. The plant was built by Lummus Construction but the owners went bankrupt before it was ever in full operation. The U.S. Department of Energy, who had guaranteed a $70 million loan for the company, has taken possession and is attempting to resell the facility.

Use of alcohols in fuels is of interest in countries around the world. Brazil leads the world in the usage of ethanol for fuel. More than 8 billion litres per year are made each year for fuel use in Brazil. We recently met with a Japanese delegation of companies who are sponsoring research and development of technologies for the production of fuel ethanol. European countries have been testing and researching neat alcohols and alcohol blends. Most of those countries have concentrated on methanol or a methanol/higher alcohol blend. Methanol has the advantage of being significantly less expensive to produce than ethanol. Ethanol does however have a major advantage over methanol in that the phase separation problem (water in fuel causing alcohol and gasoline to separate) is much less severe with ethanol.

Although other countries, except Brazil, seem to be concentrating on methanol, we are confident that the market will continue to grow for fuel ethanol. We anticipate that the U.S. Federal subsidy will be extended past 1992 and this will give impetus to an increase in fuel ethanol demand. We anticipate no decline in fuel ethanol use but expect to see a growth in the U.S. from the current 1 billion gallons per year.

PROTECTION

Our U.S. patent issued September 12, 1989 as #4,865,973. We assess our protection as broad and strong and note that it also covers our process for other applications in addition to ethanol production. We have filed patent applications in Canada, Japan, Britain, Germany, Sweden and France. A copy of our U.S. patent is shown as Appendix 10.

COMMERCIALIZATION

Our process is well engineered and considerably more developed than most university-based technologies. The process has however been developed only on a laboratory scale and pilot plant scale-up is needed to obtain sufficient engineering data on which to base the construction of a new plant or the design of a large scale retrofit. PARTEQ has now introduced the technology to major ethanol producers and design engineering firms in Canada and abroad. Although the response has been very positive, our efforts have been hampered by the fact that the technology has not yet been proven on a larger scale and by the reluctance of the industry to do anything, at least in North America, until the tax incentive is extended.

We have contacted the major ethanol plant construction design firms such as Vogelbusch and Raphael Katzen & Associates but all have declined involvement with the technology at this time. Monsanto EnviroChem, a design engineering construction firm, have indicated a desire to acquire and exploit our technology. We have held a number of discussions with EnviroChem officials and although they clearly favour our technology over other fermentation technologies, they are not prepared to commit funds to acquire the technology until the tax legislation is passed. No major Canadian engineering design firms are extensively involved in ethanol plant construction and the design firms that we did approach declined involvement at this stage.

We have contacted numerous ethanol producers but with the future of the ethanol industry hanging on whether favourable tax legislation is extended, no one is prepared to commit to any new technologies, and hence capital expenditures. We have presented the technology to major ethanol producers such as Archer-Daniels-Midland, A.E. Staley, Grain Processing Corporation, Chematur AB, Codistil, New Energy, Minnesota Corn Processors and Nofsinger. In an effort to get the technology into an actual plant environment, we have been working with a small ethanol producer, Jon Ton Alcohol Inc., in Edinburgh, Texas. They are interested in adopting our technology on a retrofit basis as a means of increasing their capacity. We are currently working with Monsanto EnviroChem to provide an estimate of the cost of the retrofit. If Jon Ton accepts the proposal, we will supply Jon Ton with a site specific license at no cost and Monsanto EnviroChem will do the engineering at cost. Although such an installation will show the value of our process in a real plant situation, a pilot plant will still be needed in order to do further development and provide sufficient data for a more sophisticated new plant designs and retrofits.

We have presented the technology to groups, such as Davis Engineering in Ottawa, which we feel be interested in pursuing a joint venture route. We have also presented a joint venture business plan to some venture capital groups but no positive responses have yet been received.

We have presented the technology as well to foreign groups from Sweden, Denmark, Australia, Brazil, Germany, the Dominican Republic and Japan. We are still cultivating a lot of these relationships and hope to have some success in one or more of these countries.

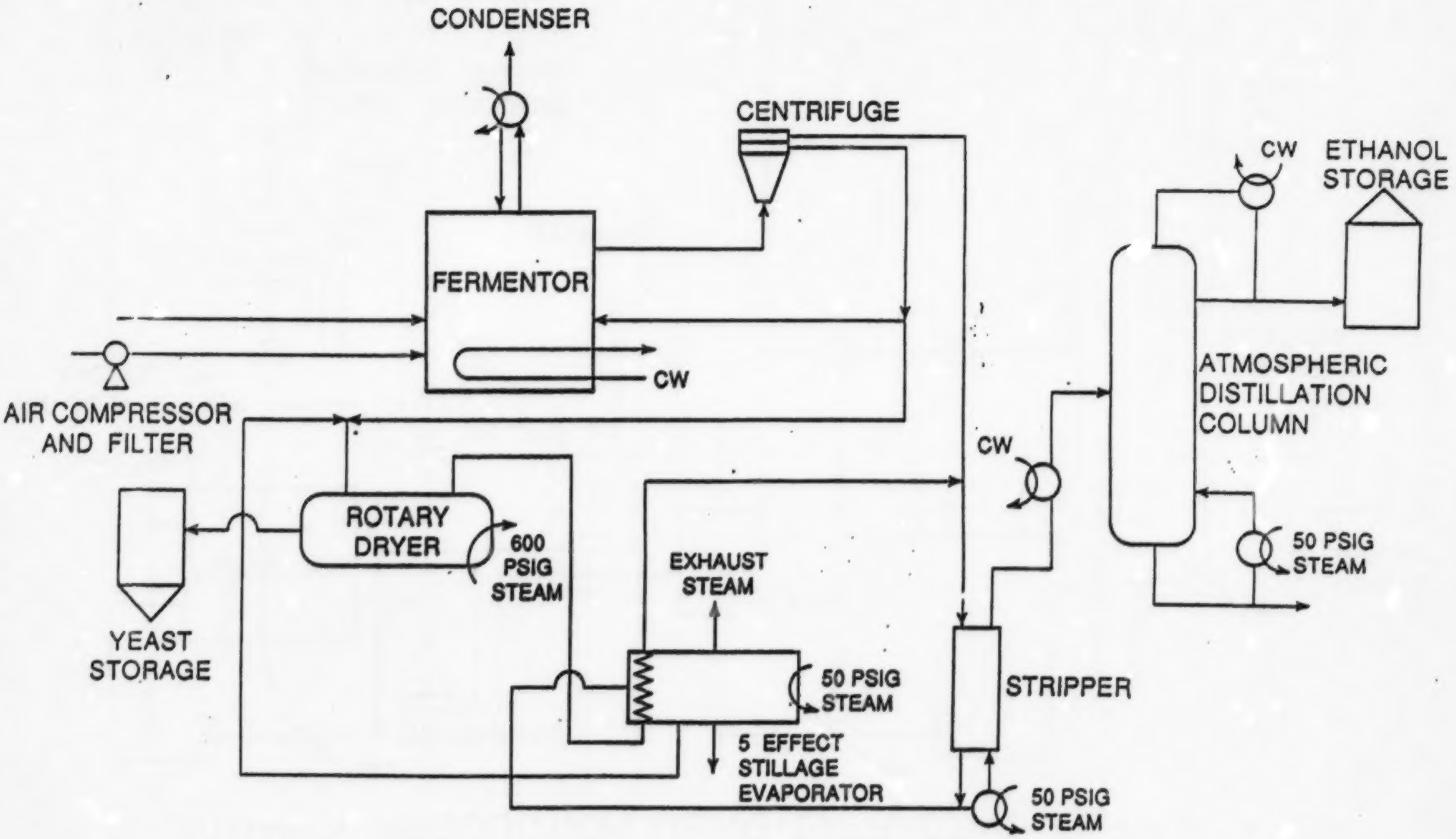
A technology information package has been prepared. The technology has also been described in trade news letters and has been presented at scientific and technology transfer meetings. We shall be presenting the technology at the next international meeting on Fuel Alcohols and Chemicals from Biomass in January 1991.

PARTEQ is continuing to explore all possible commercialization options but no final commercialization strategy has yet been determined. The actual commercialization strategy could range from the establishment of a new venture and the actual building of an ethanol production plant to exclusive licensing of the technology to one ethanol producer who may wish to use the technology advantage to undercut price and gain market share. The most important aspect of the commercialization strategy at this point in time is that we must attract the necessary funds to build a pilot plant facility so that more economic and technical information can be obtained. Once the pilot plant information becomes available and the market situation begins to unfold, a more definite commercialization strategy will have to be developed.

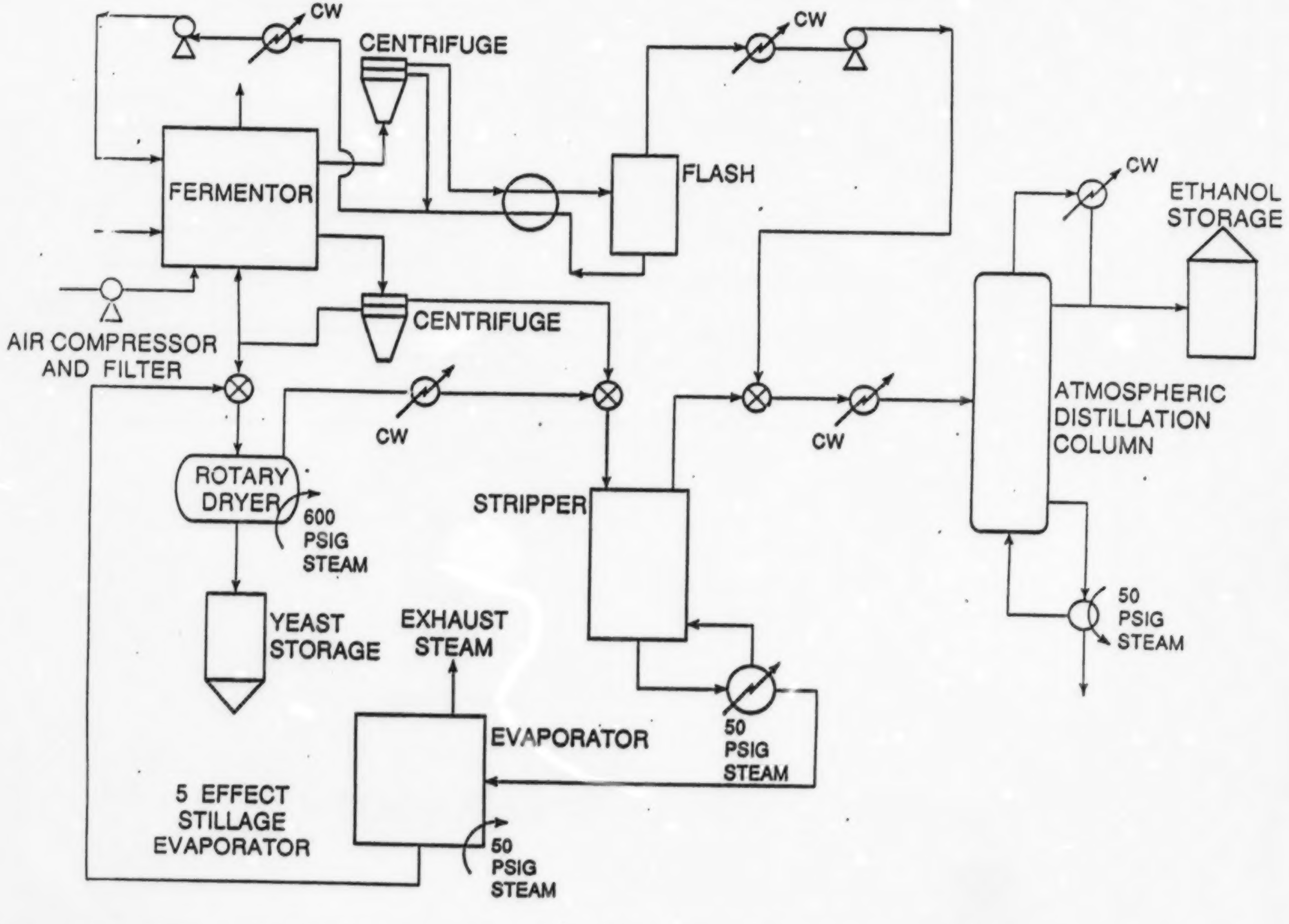
It is however, clear that the technology is of considerable value to the ethanol industry. Return on any commercialization scenario will be high. For example, we expect that if we are successful with straight licensing alone, our technology will generate revenues in excess of $5,000,000 over the first five years after the technology is introduced.

APPENDIX 1

CONVENTIONAL ETHANOL PRODUCTION


CONDENSER
CENTRIFUGE
CW
ETHANOL STORAGE
FERMENTOR
CW
AIR COMPRESSOR AND FILTER
ATMOSPHERIC DISTILLATION COLUMN
CW
ROTARY DRYER
600 PSIG STEAM
EXHAUST STEAM
50 PSIG STEAM
YEAST STORAGE
50 PSIG STEAM
STRIPPER
5 EFFECT STILLAGE EVAPORATOR
50 PSIG STEAM

ETHANOL PRODUCTION USING EXTRACTIVE FERMENTATION



CW
CENTRIFUGE
CW
FERMENTOR
FLASH
CW
ETHANOL STORAGE
CENTRIFUGE
AIR COMPRESSOR AND FILTER
CW
CW
ATMOSPHERIC DISTILLATION COLUMN
ROTARY DRYER
600 PSIG STEAM
STRIPPER
50 PSIG STEAM
YEAST STORAGE
EXHAUST STEAM
EVAPORATOR
50 PSIG STEAM
5 EFFECT STILLAGE EVAPORATOR
50 PSIG STEAM

APPENDIX 2

Assumptions Used In PROCESS Ethanol Plant

FERMENTOR

1. Cell Concentration	75 g/L
2. Maximum ethanol concentration	87.5 g/L
3. Extent of equilibrium	100 %
4. Head room	10 % of total volume
5. Solvent room	10 % of liquid volume
6. Overall heat transfer coefficient	1135 $W/m^2\cdot$deg C
7. Temp. cooling water in	10 deg C
8. Temp. cooling water out	25 deg C
9. Fermentor temperature	30 deg C
10. Fermentor agitator power	0.5 kW/m^3
11. Maximum fermentor volume	200 m^3
12. Kinetic Model	$\nu=\nu_{max}\cdot S/(K_s+S)\cdot(1-P/P_m)^n$
13. Value of toxic power coefficent (n)	0.75
14. Solids (unfermentable) conc. in feed	5 % (wt)
15. Distribution coefficient	0.25

CENTRIFUGE

1. Power requirement	0.0011 kW/kg flow
2. Concentration factor	0.2

EVAPORATOR

1. Concentration of solids in evaporator	30 % (wt)
2. Overall heat transfer coefficient	2271 $W/m^2\cdot K$
3. Boiling point rise from glucose	Not taken into account
4. PROCESS model	Simple heat exchanger
5. Heat source	50 psig steam
6. Exhaust steam	25% of total exhaust stream

ROTARY DRYER

1. Power requirement	0.0059 kW/kg evaporate flow
2. Moisture content of solids	8 % (wt)
3. PROCESS model	Simple heat exchanger
4. Heat source	600 psig steam

DISTILLATION COLUMN

1. Number of trays	40 + 1 reboiler + 1 total condenser
2. Operating pressure	Atmospheric pressure
3. Ethanol concentration overhead	93.5 % (wt)
4. Ethanol recovery	99.5 % (wt)

STRIPPER

1. Number of theoretical trays	15 + 1 reboiler
2. Ethanol concentration overhead	approximately 44 % (wt)
3. Operating pressure	Atmospheric pressure
4. Ethanol concentration in bottoms	0 % (wt)

RAW MATERIALS

1. Fermentables cost	0.15 $/kg
2. Solvent cost	4.75 $/kg
3. Dried yeast credit	0.537 $/kg
4. Solid debris credit	0.195 $/kg

OPERATIONS

1. Operating labour	6 people/shift
2. Wages	18 $/person/hour
3. Supervisory/clerical	15 % of operating labour
4. Maintenance/repairs	4 % of fixed investment capital
5. Operating supplies	15 % of maintenance/repairs
6. Laboratory expenses	15 % of operating labour
7. Overhead	60 % of operating, maintenance, and supervisory labour
8. Property tax	2 % of fixed investment capital
9. Insurance	1 % of fixed investment capital
10. Administration	25 % of operating labour
11. Distribution	10 % of total manufact. expense
12. Plant life	25 years
13. Salvage value	0 $
14. Return on investment (after tax)	15 % of total capital investment
15. MS index	900
16. Total delivered cost	1.15 x total purchased equip. cost
17. Total fixed capital	4.04 x total delivered cost
18. Total working capital	10 % of total investment
19. Inflation	5 %/year
20. Income tax	50 % of net profit before tax
21. Green-fields plant construction period	2 years
22. Total Working Capital	INCLUDES COSTS FOR: a. 1 month's stock of raw materials and supplies at delivered prices b. 1 month's manufacturing costs for finished product in stock c. Accounts receivable (1 month's production costs, salaries, and wages) and taxes payable
23. Production period	330 days
24. Plant capacity	100 million L/yr azeotropic ethanol
25. Depreciation	Straight line

UTILITIES

1. Atmospheric steam	0.00258 \$/kg
2. Low pressure steam (50 psig)	0.01336 \$/kg
3. High pressure steam (600 psig)	0.01426 \$/kg
4. Cooling water	0.312 $\$/m^3$
5. Line losses (excluding elec.)	5 % added
6. Electrical line losses and lighting	20 % added
7. Electricity costs:	\$ 0.095/kWh for <250 kWh/month
	\$ 0.0584/kWh for <12250 kWh/month
	\$ 0.0417/kWh for <2080000 kWh/month
	\$ 0.025/kWh for >2080000 kWh/month

EQUIPMENT COST CORRELATIONS

Item	Unit Cost Correlation	Capacity Units
Storage Tank	$\text{MSI} \times 0.205 \times \text{Capacity}^{0.48}$	L
Fermentor	$\text{MSI} \times 0.204 \times \text{Capacity}^{0.55}$	L
Fermentor Agitator	$\text{MSI} \times 6.236 \times \text{Capacity}^{0.67}$	kW
Fermentor Cooler	$\text{MSI} \times 2.372 \times \text{Capacity}^{0.32}$	m^2
Air Filter	$\text{MSI} \times 0.007 \times \text{Capacity}^{0.67}$	kg gas/hr
Air Compressor	$\text{MSI} \times 8.782 \times \text{Capacity}^{0.32}$	kg gas/hr
Centrifuge	$\text{MSI} \times 0.022 \times \text{Capacity}^{0.86}$	kg feed/hr
Rotary Dryer	$\text{MSI} \times 19.63 \times \text{Capacity}^{0.47}$	kg evaporate/hr
Stripper/Distillation	$\text{MSI} \times 109.9 \times \text{Capacity}^{0.14}$	kg water/hr
Stillage Evaporator	$\text{MSI} \times 4.752 \times \text{Capacity}^{0.55}$	kg feed/hr
Flash Unit	$\text{MSI} \times 0.222 \times \text{Capacity}^{0.55}$	L

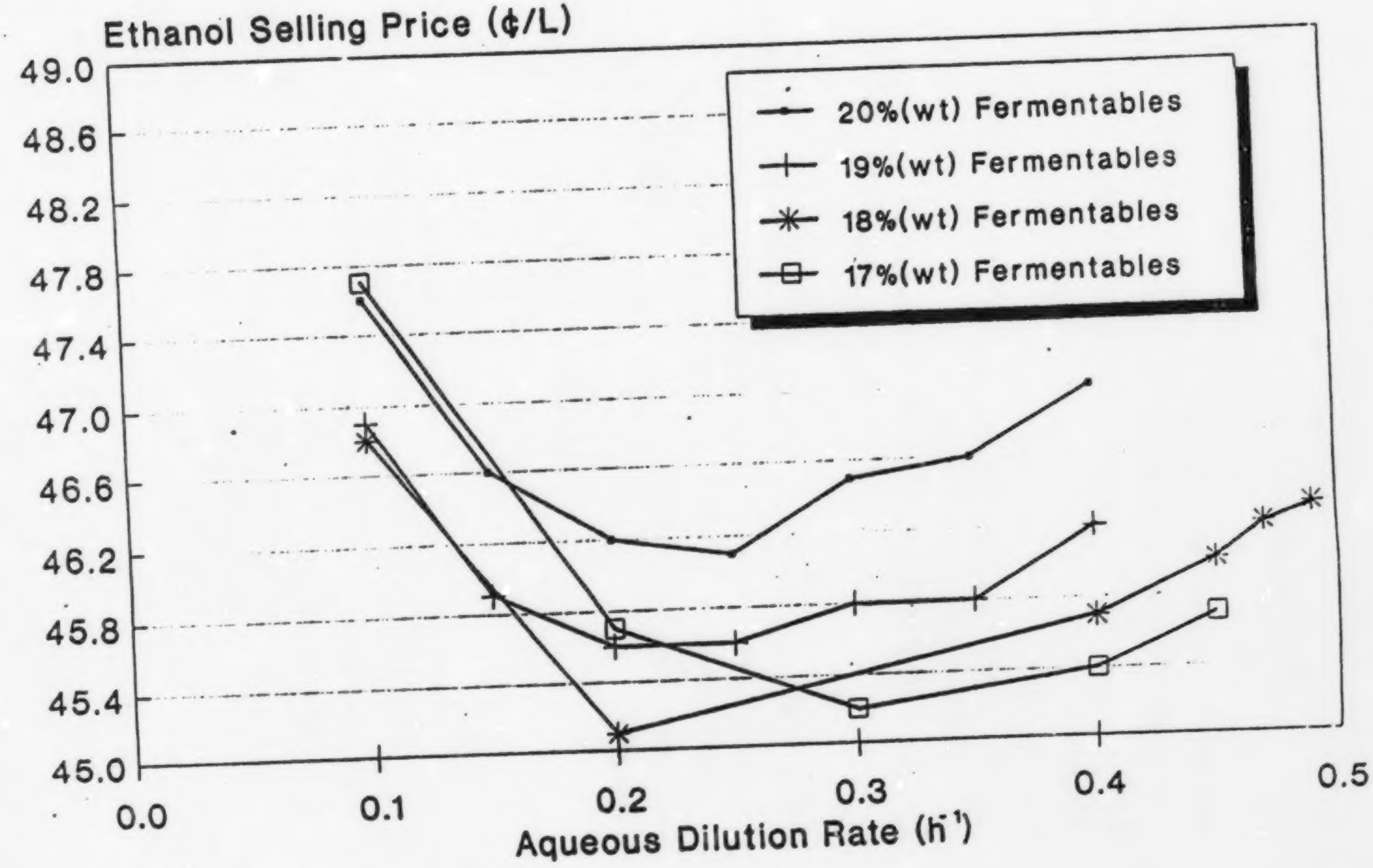
FACTORS TO CONVERT DELIVERED EQUIPMENT COSTS TO FIXED CAPITAL INVESTMENT

DETAILS		CONVERSION FACTORS FOR A GREEN-FIELDS FACILITY
Delivered Equipment (not including pumps)		1.00
Installation (including foundations)		1.04
Piping		0.33
Electrical		0.09
Instrumentation		0.13
Battery Limits Building and Services (indoor facility)		0.45
Land (includes pumps, land, yard improvements)		0.06
	Total Physical Plant	3.10
Engineering		0.41
	Direct Plant Cost	3.51
Contractor Fees		0.17
Contingency		0.36
	Total Fixed Capital	4.04 x Delivered Cost

APPENDIX 3

OPTIMUM OPERATING CONDITIONS

Conventional Fermentation (Green Fields)



APPENDIX 4

Economic Results for Conventional Fermentation, Green Fields
Run CF07
Production - 98,539,056 L/yr Azeotropic Ethanol
October 25, 1989

COSTS

Purchased Equipment:

Storage	$444,513
Fermentation	$1,669,440
Dryer	$1,048,560
Stripper	$504,311
Evaporator	$2,407,490
Total Purchased Equip.	$6,074,314
Total Delivered Equip.	$6,985,461
Total Fixed Capital Invest.	$28,221,263
Total Working Capital	$2,822,037
Total Capital Investment	$31,043,300

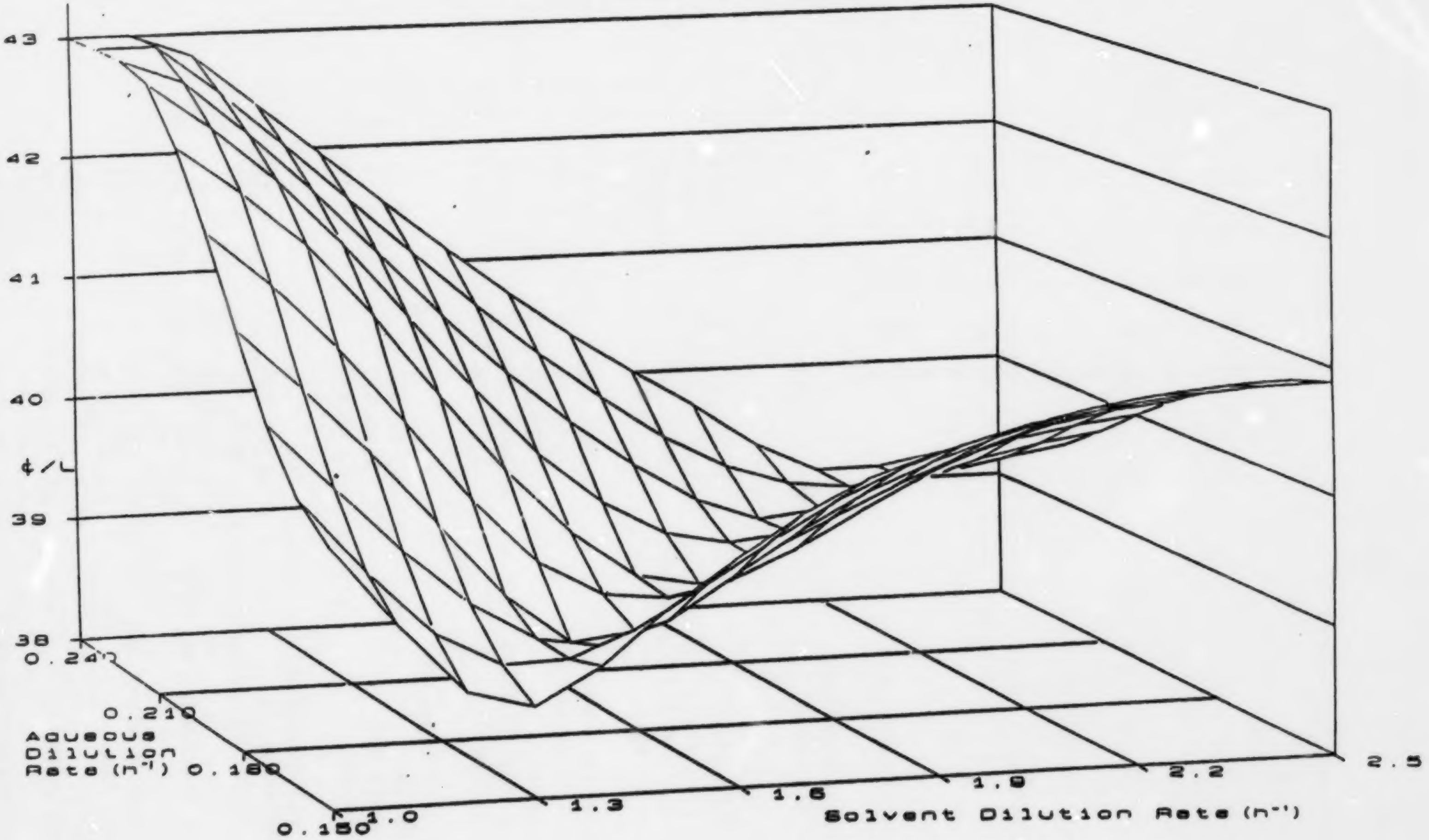
MANUFACTURING EXPENSES

Direct Expenses:	$/yr	cents/L
Raw Materials	$26,617,700	27.01
Utilities		
50 psig steam	$9,325,166	9.46
600 psig steam	$1,034,748	1.05
Process and cooling water	$706,128	0.72
Electricity	$490,776	0.50
Operating Labour	$855,360	0.87
Supervisory Labour	$128,304	0.13
Maintenance	$1,128,851	1.15
Operating Supplies	$169,328	0.17
Laboratory Expenses	$128,304	0.13
Indirect Expenses:		
Overhead	$1,267,509	1.29
Property Tax	$564,425	0.57
Insurance	$282,213	0.29
General Expenses:		
Administration	$213,840	0.22
Distribution/marketing	$4,269,880	4.33
Total Production Expense	$47,182,532	47.88
Net Profit After Tax	$4,656,490	4.73
Income Tax	$4,656,490	4.73
Credits	($13,337,300)	-13.54
Depreciation	$1,241,732	1.26
Total Production Cost	$44,399,944	45.06

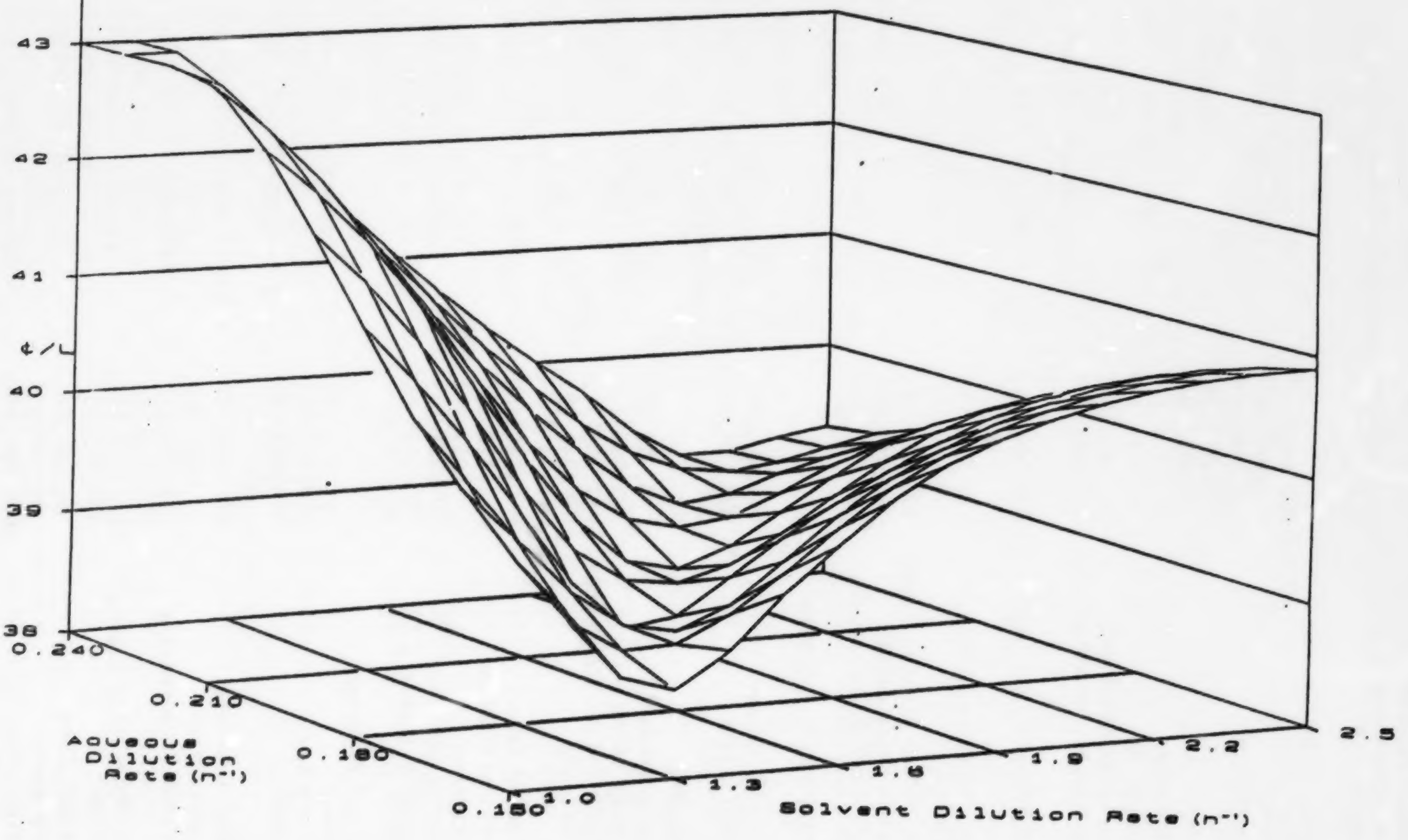
APPENDIX 5

Optimum Operating Conditions For Extractive Fermentation (green fields)

Fermentables in Feed = 500 g/l (41.5 wt. %)



Optimum Operating Conditions
For Extractive Fermentation (green fields)
Fermentables in Feed = 500 g/l (41.5 wt. %)



APPENDIX 6

Economic Results for Extractive Fermentation, Green Fields
Run EF15
Production - 98,150,976 L/yr Azeotropic Ethanol
October 25, 1989

COSTS

Purchased Equipment:

Storage	$494,885
Fermentation	$776,954
Dryer	$1,029,350
Stripper	$425,538
Evaporator	$1,240,120
Flash Unit	$156,342
Centrifuge (aqueous)	$198,401
Centrifuge (solvent)	$1,063,790
Total Purchased Equip.	$5,385,380
Total Delivered Equip.	$6,193,187
Total Fixed Capital Invest.	$25,020,475
Total Working Capital	$2,943,363
Total Capital Investment	$27,963,838

MANUFACTURING EXPENSES

Direct Expenses:	**$/yr**	**cents/L**
Raw Materials	**$26,324,500**	**26.82**
Utilities		
50 psig steam	**$4,317,105**	**4.40**
600 psig steam	**$1,000,811**	**1.02**
Process and cooling water	**$1,205,812**	**1.23**
Electricity	**$341,322**	**0.35**
Operating Labour	**$855,360**	**0.87**
Supervisory Labour	**$128,304**	**0.13**
Maintenance	**$963,948**	**0.98**
Operating Supplies	**$144,592**	**0.15**
Laboratory Expenses	**$128,304**	**0.13**
Indirect Expenses:		
Overhead	**$1,168,567**	**1.19**
Property Tax	**$481,974**	**0.49**
Insurance	**$240,987**	**0.25**
General Expenses:		
Administration	$213,840	0.22
Distribution/marketing	$3,730,159	3.80
Total Production Expense	$41,245,585	42.02
Net Profit After Tax	$4,194,570	4.27
Income Tax	$4,194,570	4.27
Credits	($12,774,700)	-13.02
Depreciation	$1,118,554	1.14
Total Production Cost	$37,978,579	38.69

APPENDIX 7

Economic Comparison of RETRONEW To Conventional Plant
(Conventional Production - 98,539,056 L/a Azeotropic Ethanol)
(Retrofitted Production - 254,882,000 L/a Azeotropic Ethanol)

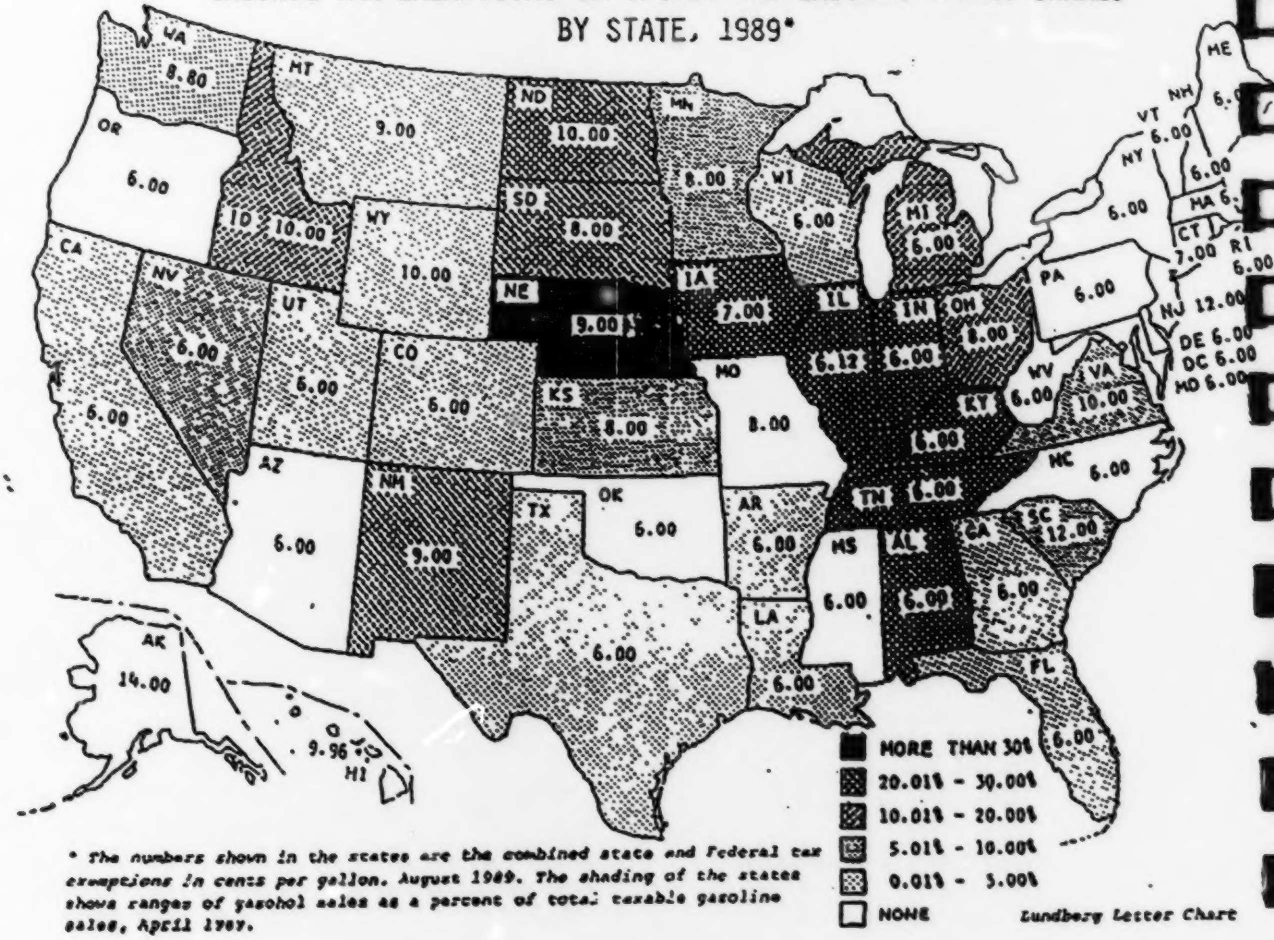
COSTS ($ CAN)	Conventional Plant		Retrofitted Plant	
Purchased Equipment:				
Storage	$445,000		$550,000	
Fermentation	$1,669,000			
Dryer	$1,049,000		$1,230,000	
Stripper	$504,000			
Evaporator	$2,407,000			
Centrifuge/Flash			$2,917,000	
Solvent			$1,188,000	
Total Purchased Equip.	$6,074,000		$5,885,000	
Total Delivered Equip.	$6,985,000		$6,768,000	
Total Fixed Capital Invest.	$28,221,000			
Total Working Capital	$2,822,000			
Total Capital Investment	$31,043,000		$6,768,000	
MANUFACTURING EXPENSES				
Direct Expenses:	$/a	cents/L	$/a	cents/L
Raw Materials	$26,618,000	27.01	$68,865,000	27.02
Utilities				
50 psig steam	$9,325,000	9.46	$11,581,000	4.54
600 psig steam	$1,035,000	1.05	$2,520,000	0.99
Process and cooling water	$706,000	0.72	$3,280,000	1.29
Electricity	$491,000	0.50	$861,000	0.34
Operating Labour	$855,000	0.87	$855,000	0.34
Supervisory Labour	$128,000	0.13	$128,000	0.05
Maintenance	$1,129,000	1.15	$1,129,000	0.44
Operating Supplies	$169,000	0.17	$169,000	0.07
Laboratory Expenses	$128,000	0.13	$128,000	0.05
Indirect Expenses:				
Overhead	$1,268,000	1.29	$1,268,000	0.50
Property Tax	$564,000	0.57	$564,000	0.22
Insurance	$282,000	0.29	$282,000	0.11
General Expenses:				
Administration	$214,000	0.22	$214,000	0.08
Distribution/marketing	$4,270,000	4.33	$4,270,000	1.68
Total Production Expense	$47,183,000	47.88	$96,115,000	37.71
Net Profit After Tax	$4,656,000	4.73	$4,656,000	1.83
Income Tax	$4,656,000	4.73	$5,671,000	2.22
Credits	($13,337,000)	-13.54	($34,194,000)	-13.42
Depreciation	$1,242,000	1.26	$1,782,000	0.70
Additional ROI			$1,015,000	0.40
Total Production Cost	$44,400,000	45.06	$75,045,000	29.44

APPENDIX 8
GASOHOL TAX EXEMPTIONS & MARKET SHARES

The Original Alternative

GASOHOL: A BLEND OF INCENTIVES

GASOHOL TAX EXEMPTIONS IN ¢/GAL. AND GASOHOL MARKET SHARES
BY STATE, 1989*


WA 8.80
OR 6.00
CA 6.00
NV 6.00
ID 10.00
MT 9.00
WY 10.00
UT 6.00
AZ 6.00
CO 6.00
NM 9.00
ND 10.00
SD 8.00
NE 9.00
KS 8.00
OK 6.00
TX 6.00
MN 8.00
IA 7.00
MO 8.00
AR 6.00
LA 6.00
WI 6.00
IL 6.12
MS 6.00
MI 6.00
IN 6.00
KY 6.00
TN 6.00
AL 6.00
OH 8.00
GA 6.00
FL 6.00
SC 12.00
NC 6.00
VA 10.00
WV 6.00
PA 6.00
NY 6.00
VT 6.00
NH 6.0
ME
MA 6.
CT 7.00
RI 6.00
NJ 12.00
DE 6.00
DC 6.00
MD 6.00
AK 14.00
HI 9.96
■ MORE THAN 30%
▩ 20.01% - 30.00%
▨ 10.01% - 20.00%
▦ 5.01% - 10.00%
▒ 0.01% - 5.00%
☐ NONE
Lundberg Letter Chart

** The numbers shown in the states are the combined state and Federal tax exemptions in cents per gallon, August 1989. The shading of the states shows ranges of gasohol sales as a percent of total taxable gasoline sales, April 1989.*

APPENDIX 9
FUEL ETHANOL PRODUCERS - U.S.

David Swartzentruber
Rt 1. Box 50
Greenwood DE 19950
302-349-4896

Wiles Fuel
RR #1 Box 46
West Union, IL 62477
217-279-3321

Webster Enterprises
RR #1 Box 201
Golden Eagle, IL 62036
618-883-2240

Young Fuel Distillery
RR #4 Box 10
Rushville, IL 62681
217-322-3888

Richard Fagen
Route 3
Granite Falls, MN 56241
612-564-3152

Lee Bryant
Route 3 Box 288
Willmar, MN 56201
612-235-5570

Bernard Nietfield
Route 1
Melrose, MN 56352
612-987-3442

Gary Thulien
Route 2 Box 202
Windom, MN 56101
507-831-1578

Southwest Montana Alcohol Processors
310 E. Sabree
Dillon, MT 59725
406-683-6113

Geoff Schneider
Route 1
Walton, NE 68461
402-781-2232

George Dudley
Route 1 Box 147
Reidsville, NC 27320

Kreider Farms
Rt. 1 Box 165
Cochranville, PA 19330
215-932-4700

Swisher Electric Cooperative
Box 127
Tulia, TX 79088
806-995-3567

Parallel Products, Inc.
12281 Arrow Rt
Cucamonga, CA 91739
714-980-1200
(uses industrial waste)

Golden Cheese Company of California
1138 W. Rincon St.
Corona, CA 91720
714-737-9260
(uses cheese whey)

Dairymen's Cooperatives
400 S. M St.
Tulare, CA 92374
209-685-68000
(uses cheese whey)

Ag Power of Colorado
13255 Colorado Blvd. 501-B
Denver, CO
303-989-3343
(uses brewery waste)

Pacific Ethanol Products
91-259 Olai St.
Ewa Beach, HI 96707
808-682-4433
(uses molasses)

J.R. Simplot Company
P.O. Box 130
Burley, ID 83318
208-678-3571
(uses potato waste)

Archer Daniels Midland
P.O. Box 1470
Decatur, IL 62525
217-424-5200

Vienna Correctional Center
P.O. Box 200
Vienna, IL 62995
618-658-8371

Pekin Energy
Box 10
Pekin, IL 61555
309-347-9380

Midwest Grain Products, Inc.
P.O. Box 1069
Pekin, IL 61554
309-333-3990

Archer Daniels Midland
Foot of Edmund Street
Peoria, IL 61601
309-673-7828

New Energy Company of Indiana
320 W. Calvert St.
South Bend, IN 46680
219-233-3116

Grain Processing Corporation
1600 Oregon Street
Muscatine, IA 52761
319-264-4286

Archer Daniels Midland
P.O. Box 340
Clinton, IA 52732
3319-244-5257

Manildra Energy, Inc.
32 SE Main St., Ste. OH 508
Minneapolis, MN 55414
612-382-7408

High Plains Corporation
333 N. Waco St.
Wichita, KS 67202
316-269-4310

Reeve AgriEnergy
Box 1036
Garden City, KS 67846
316-275-7541

ESE Alcohol, Inc.
Box 813
Leoti, KS 67861
316-375-4904

Kraft, Inc.
2211 Sanders Rd.
Northbrook, IL 60062
312-498-8174
(uses cheese whey)

G&S Gasohol, Inc.
Hwy. 68 & 169 South Box 476
Mankato, MN 56001
507-387-4403
(no fermentation)

Minnesota Corn Processors
400 W. Main St. P.O. Box 1236
Marshall, MN 56528
507-537-0577

Graf Alcohol
4375 County Rd. 10 North
Watertown, MN 55388
612-955-1666

Alcotech
P.O. Box 154
Ringling, MT 59642
406-547-2116

American Diversified Companies
P.O. Box 488
Hastings, NE 68901
402-463-6885

Energy Fuels Development
P.O. Box 892
Portales, NM 88130
505-356-8535

Grain Power Tucumcari Ltd.
1600 Rock Island St.
Tucumcari, NM 88401
505-461-0393

West Texas Ethanol
Route 3, Box 285-A
Clovis, NM 88101
505-769-2187

A.E.I.
Route 2, Box 307-A
Clovis, NM 88101
505-762-3030

Cenex Agri-Fuels Co.
P.O. Box 700, County Rd. 9
Walhalla, ND 58282
701-549-3931

Alchem Ltd.
P.O. Box 32
Grafton, ND 58237
701-352-0602

South Point Ethanol
P.O. Box 1004
South Point, OH 45680
614-377-2765

Bowman Energy Inc.
P.O. Box 217
Pinewood, SC 29125
803-452-6469

A.E. Staley Manufacturing Company
1 Continental Powers
1701 Gold Rd., Ste. 1000
Rolling Meadows, IL 60008
312-364-8704

Jonton Alcohol, Inc.
Route 3, Box 151-E
Edinburg, TX 78539
512-842-3378
(used molasses)

A. Smith Bowman Distillery
1 Bowman Drive
Fredericksburg, VA 22401
703-373-4555
(no fermentation)

Pinedell Associates, Ltd.
Box 310
Providence Forge, VA 23140
804-829-2493
(no fermentation)

New Church Energy Associates I & II, Ltd.
P.O. Box 147
New Church, VA 23415
804-824-4400

Chemical Ethanol Producers, Inc.
Box 5097
Chesapeake, VA 23324
804-543-1321
(no fermentation)

ETOH, Inc.
Route 8, Box 66A
Wilsons, VA 23894
804-265-8300
(no fermentation)

American Fuel Trading Company
2 East Brian Street
Savannah, GA 31402
912-234-1261
(no fermentation)

Virginia Solid Fuels Inc.
Route 2, Box 388
Bealton, VA 22712
703-439-8944
(no fermentation)

Bio-Regional Energy Associates, Ltd.
Floyd Industrial Park Rd.
Floyd, VA 24091
703-745-3923
(no fermentation)

Virginia Feed & Fuel Inc.
P.O. Box 452
Madison, VA 22727
703-948-6886
(no fermentation)

Butterwood Farms
Rt. 1 102
Wilsons, VA 23894
804-265-8023
(no fermentation)

Virginia Oxygenates Inc.
3548 Lee Highway
Bristol, VA 24201
703-669-0495
(no fermentation)

Parker Oil Company
P.O. Box 310
Providence Forge, VA 23140
804-829-2493
(no fermentation)

RAJ Chemicals of Virginia, Inc.
P.O. Box 7437
Chesapeake, VA 23324
(no fermentation)

Montvale Ethanol Inc.
333 N. Waco Street
Wichita, KS 67202
316-269-4310
(no fermentation)

Georgia-Pacific Corp.
P.O. Box 1236
Bellingham, WA 98227
206-733-4410
(uses wood mill waste)

Pabst Brewing Co.
P.O. Box 947
Olympia, WA 98507
206-754-5000
(uses brewery wastes)

Grudem Brothers
Route 1, Box 103
Jim Falls, WI 54748
612-644-6911

Guy D. Griswold
Project Manager
North Carolina Ethanol, Ltd.
105 West Main St.
Faison, NC 28341-0748
919-267-1533

APPENDIX 10

The United States of America

The Commissioner of Patents and Trademarks

Has received an application for a patent for a new and useful invention. The title and description of the invention are enclosed. The requirements of law have been complied with, and it has been determined that a patent on the invention shall be granted under the law.

Therefore, this

United States Patent

Grants to the person or persons having title to this patent the right to exclude others from making, using or selling the invention throughout the United States of America for the term of seventeen years from the date of this patent, subject to the payment of maintenance fees as provided by law.

Commissioner of Patents and Trademarks

Attest

United States Patent [19]

Kollerup et al.

[45] **Date of Patent:** Sep. 12, 1989

[54] PROCESS FOR EXTRACTIVE FERMENTATION

[75] Inventors: **Finn Kollerup; Andrew J. Daugulis,** both of Kingston, Canada

[73] Assignee: **Queen's University at Kingston,** Kingston, Canada

[21] Appl. No.: 896,002

[22] Filed: **Aug. 13, 1986**

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 775,791, Sep. 13, 1985, abandoned.

[51] Int. Cl.⁴ C12P 37/00; C12P 19/04; C12P 7/48; C12P 7/06; B01D 11/04; B01D 3/34

[52] U.S. Cl. 435/43; 435/101; 435/144; 435/161; 435/813; 435/822; 435/911; 435/917; 435/935; 435/942; 435/102; 210/634; 203/60; 203/61; 203/62; 203/63; 203/64; 203/DIG. 13

[58] Field of Search 435/72, 93, 243, 101, 435/144, 150, 151, 160, 161, 162, 813, 43, 822, 911, 917, 935, 942, 102; 210/634; 203/DIG. 13, 54, 55, 60, 61; 62, 63, 64

[56] References Cited

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4,698,303	10/1987	Bailey et al.	435/139

OTHER PUBLICATIONS

Ishii et al.; *J. Chem. Eng. of Japan,* 18(2):125–130; (1985).

Taya et al., *J. Ferment. Technol.,* 63(2):181–187; (1985).

Minier et al.; *Biotech. & Bioeng.;* 24:1565–1579; (1982).

Wang et al.; *Biotech. & Bioeng. Symp.,* No. 11:555–565 (1981).

Primary Examiner—Elizabeth C. Weimar

Attorney, Agent, or Firm—Richard J. Hicks

[57] ABSTRACT

A process for producing a variety of chemical products, e.g., ethanol, by fermentation in which the product is removed from the fermentation medium as it is formed by liquid-liquid extraction using an extractant for the product which is immiscible with water. The extractant employed is chosen from the following groups: (A) double bond unsaturated aliphatic alcohols having 12 or more carbon atoms; (B) saturated branched chain aliphatic alcohols having 14 or more carbon atoms or mixtures thereof; (C) double bond unsaturated aliphatic acids having 12 or more carbon atoms; (D) aliphatic and aromatic mono-, di- or tri-esters having 12 or more carbon atoms, other than dibutyl phthalate; (E) aliphatic noncyclic ketones and aliphatic aldehydes having 12 or more carbon atoms; and (F) mixtures of extractants from groups (A) to (E) above or mixtures of at least one of the above extractants and at least one other extractant. These extractants are non-toxic to microorganisms used for fermentation, have little tendency to form emulsions and are otherwise suited for the process. The liquid-liquid extraction may thus be carried out in situ in the fermentor, preferably in a continuous stirred tank fermentor.

12 Claims, 5 Drawing Sheets

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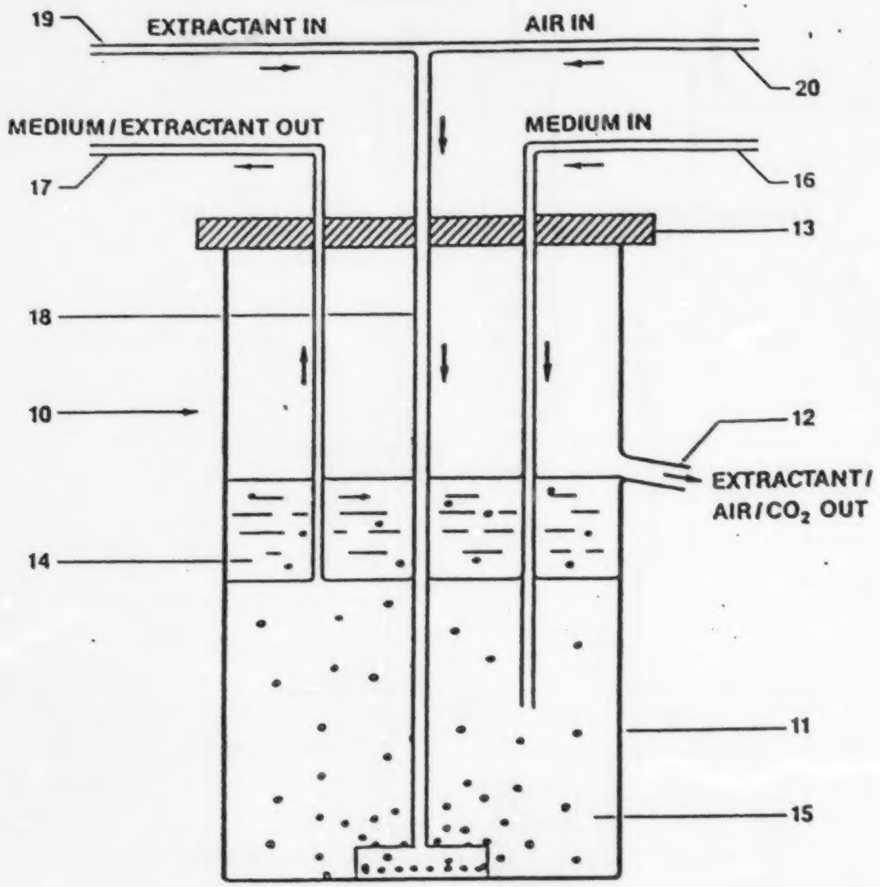
EXTRACTANT IN
AIR IN
MEDIUM/EXTRACTANT OUT
MEDIUM IN
EXTRACTANT/
AIR/CO$_2$ OUT

FIGURE 1

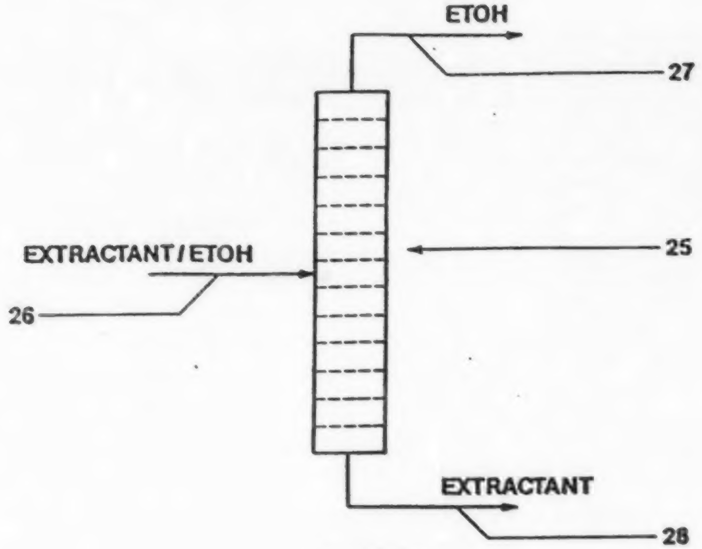
ETOH
27
25
EXTRACTANT / ETOH
26
EXTRACTANT
28

FIGURE 2

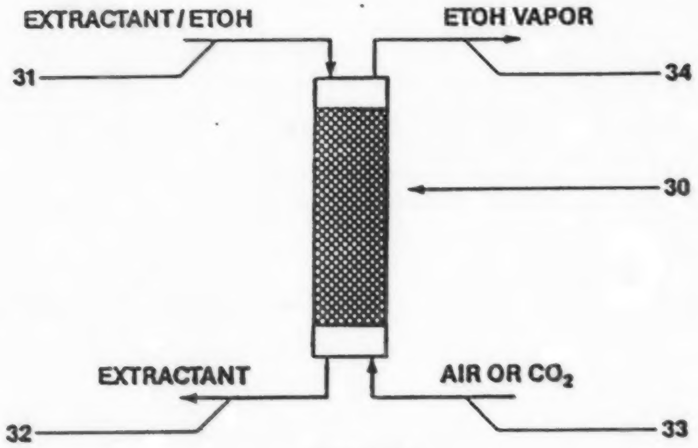
EXTRACTANT / ETOH
31
ETOH VAPOR
34
30
EXTRACTANT
32
AIR OR CO_2
33

FIGURE 3

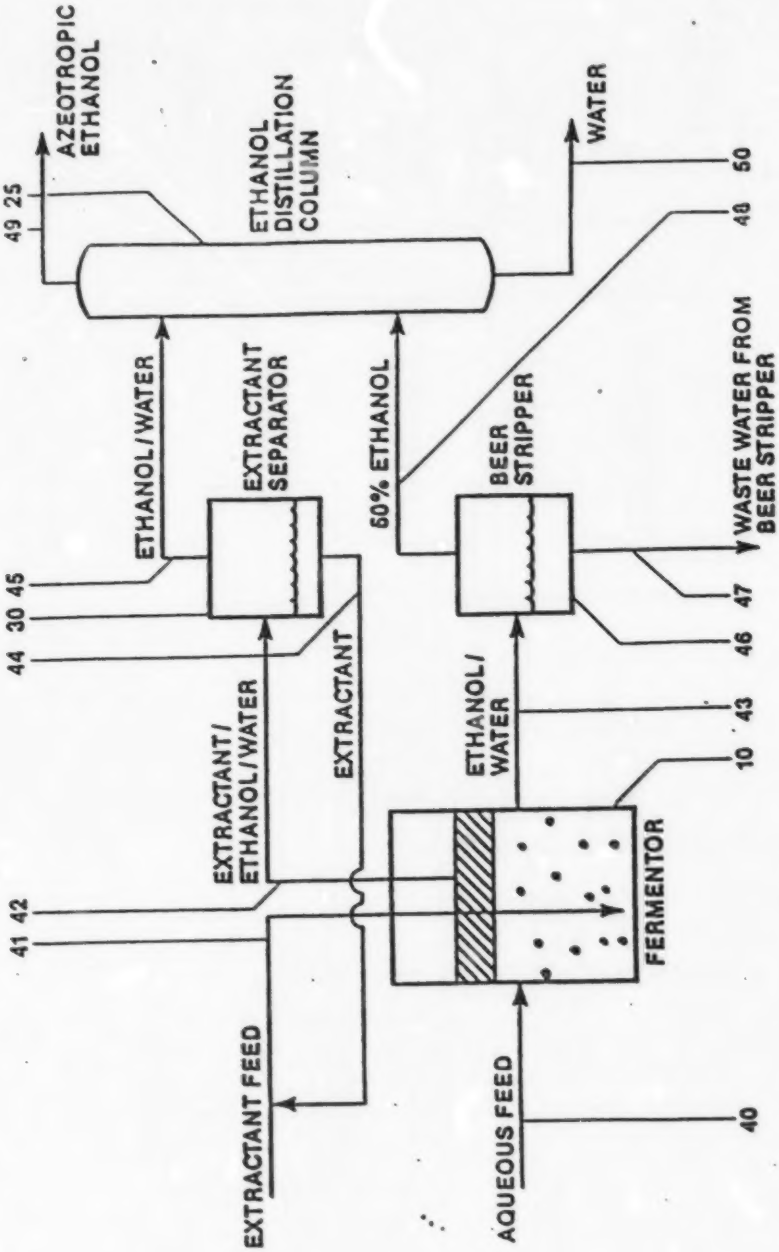
AZEOTROPIC ETHANOL
49
25
ETHANOL DISTILLATION COLUMN
WATER
50
48
ETHANOL/WATER
EXTRACTANT SEPARATOR
45
30
44
60% ETHANOL
BEER STRIPPER
WASTE WATER FROM BEER STRIPPER
47
46
EXTRACTANT/ ETHANOL/WATER
EXTRACTANT
ETHANOL/ WATER
43
10
41
42
FERMENTOR
EXTRACTANT FEED
AQUEOUS FEED
40

FIGURE 4

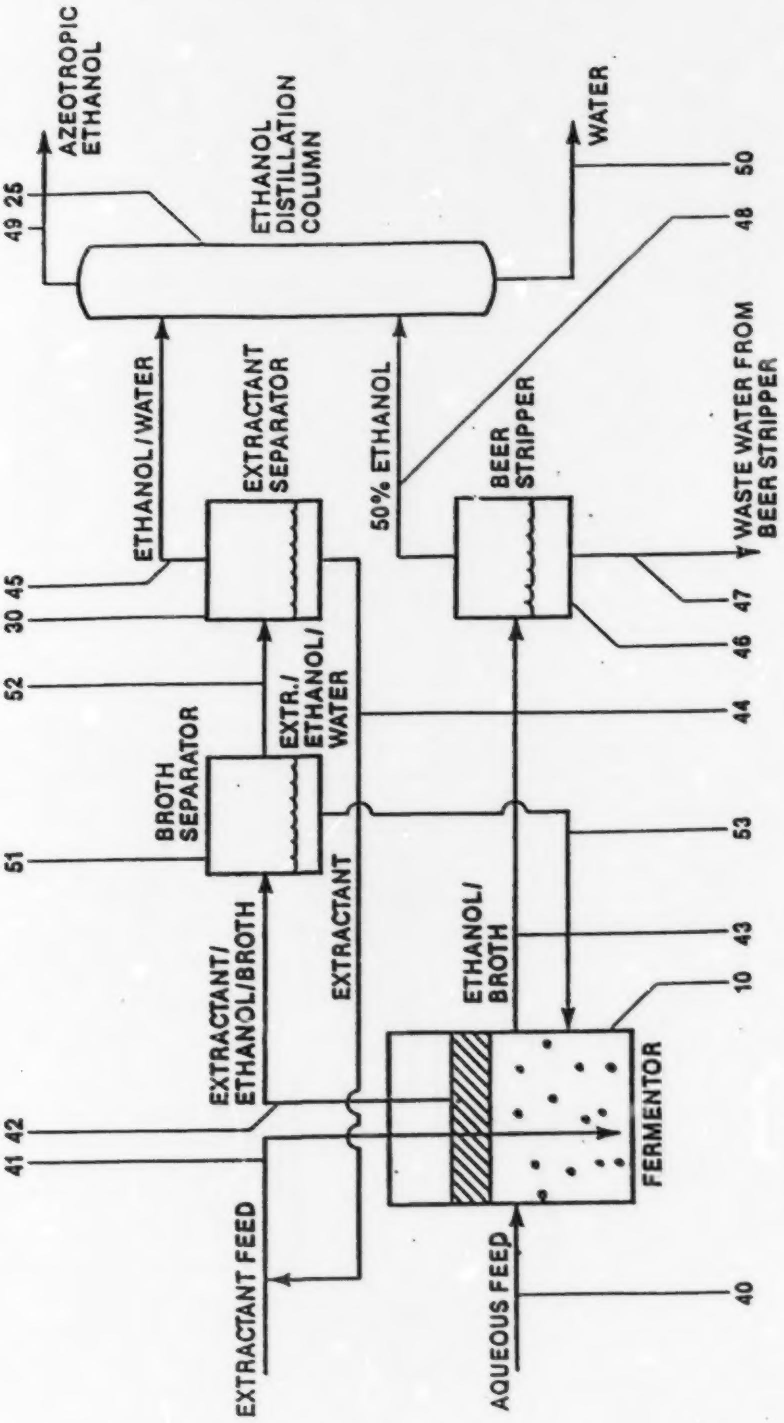
AZEOTROPIC ETHANOL
49
25
ETHANOL DISTILLATION COLUMN
WATER
50
48
ETHANOL/WATER
EXTRACTANT SEPARATOR
50% ETHANOL
BEER STRIPPER
WASTE WATER FROM BEER STRIPPER
30
45
47
46
52
EXTR./ETHANOL/WATER
44
BROTH SEPARATOR
51
53
EXTRACTANT/ETHANOL/BROTH
EXTRACTANT
ETHANOL/BROTH
43
10
42
41
FERMENTOR
EXTRACTANT FEED
AQUEOUS FEED
40

FIGURE 5

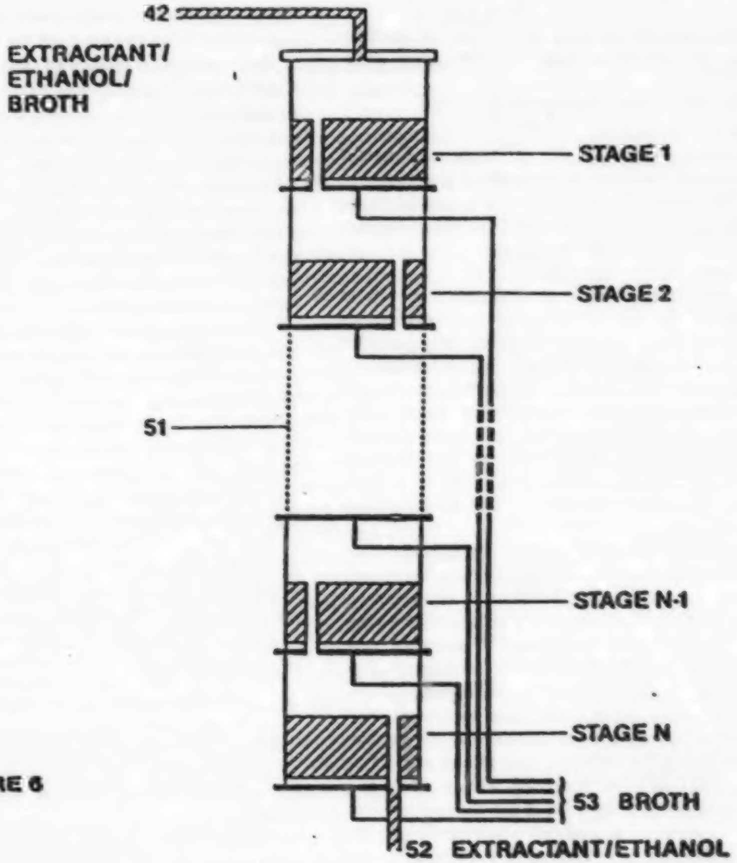
42
EXTRACTANT/ ETHANOL/ BROTH
STAGE 1
STAGE 2
51
STAGE N-1
STAGE N
53 BROTH
52 EXTRACTANT/ETHANOL

FIGURE 6

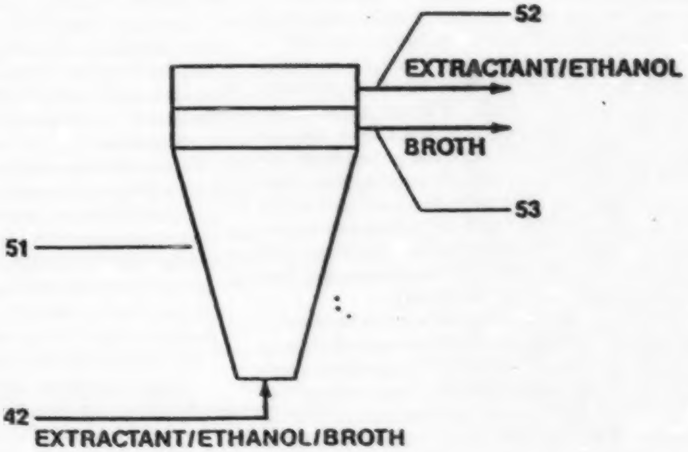
52
EXTRACTANT/ETHANOL
BROTH
53
51
42
EXTRACTANT/ETHANOL/BROTH

FIGURE 7

PROCESS FOR EXTRACTIVE FERMENTATION

This is a continuation-in-part of U.S. patent application Ser. No. 775,791, filed Sept. 13, 1985, now abandoned.

BACKGROUND OF THE INVENTION

I. Field of the Invention

This invention relates to the production of useful chemical products by fermentation. More particularly, the invention relates to fermentation procedures in which the products may be removed from the fermentation medium by liquid-liquid extraction.

II. Description of the Prior Art

It is well known that a variety of chemical products can be prepared by the culturing or fermentation of microorganisms. For example, a number of antibiotics (such as penicillin), acetone/butanol, citric acid, and, of course, ethanol may be produced in this way. A review of various techniques employed for fermentation using the preparation of ethanol as an example is provided in "Biotechnology Report", Biotechnology and Bioengineering, Vol. XXVI, pp. 1003 to 1025 (1984) by Maiorella et. al., the disclosure of which is incorporated herein by reference.

The most significant disadvantage of fermentation procedures is that the product is normally obtained only in dilute form, i.e. as a dilute solution in the aqueous fermentation medium. This is often because of the phenomenon of "end-product inhibition". That is, the rate of production of the product by the microorganism decreases as the product concentration increases, and the microorganism becomes inactivated by the product when the product reaches a certain critical concentration in the fermentation medium. For this reason ethanol, for example, can be obtained by fermentation at a concentration of no greater than about 11-12% (w/v). It is therefore necessary to provide additional steps for the concentration and purification of the product, and such steps are often difficult and expensive. In the case of ethanol, the fermentation medium is normally subjected to high cost aqueous distillation and (when absolute alcohol is required) further steps have to be taken to free the ethanol from the azeotrope it forms with water. Similar difficulties are encountered for other products.

In order to overcome the above drawback, attempts have been made to remove the product from the fermentation medium as the fermentation proceeds so that the product never reaches a harmful or critical concentration. In this way, the microorganism can function for a prolonged period of time at a high production rate. Moreover, by suitably choosing the method of removal of the product, the difficulty and expense of distilling dilute aqueous solutions may be avoided.

One such attempt utilizes a liquid which is immiscible with the aqueous fermentation medium but which is an extractant for the desired product. The product partitions between the extractant and the fermentation medium when the two are brought into contact, so that the concentration of the product in the aqueous fermentation medium is reduced. In practice, however, a number of difficulties are encountered with this process. For example, common water-immiscible solvents are toxic to most microorganisms and so cannot be used for direct contact with an aqueous fermentation medium containing microorganisms. Secondly, stable emulsions are often formed between the fermentation medium and certain extractants and this gives rise to difficulties of

separation, blocking of the equipment etc. It is also difficult to find extractants which have a high partition coefficient for the product and which can later undergo separation from the product by inexpensive techniques.

For example Gyamerah and Glover ("Ethanol by Continuous Fermentation using a Combination of Immobilized Yeast and Solvent Extraction," a paper presented at "Advances in Fermentation '83", Chelsea College, London (U.K.) Sept. 21-23, 1983) used n-dodecanol, tributyl phosphate and n-dodecane as extractants, but found that stable emulsions were formed. They attributed the problem of emulsion formation to the presence of yeast cells in the fermentation medium and so attempted to overcome the problem by immobilizing the yeast cells.

Other extractants which have been suggested are straight chain paraffin hydrocarbons (R. K. Finn "Inhibitory Cell Products: Their Formation and Some New Methods of Removal", J. Ferm. Technol. Vol. 44, p. 305-310, 1966); long chain saturated aliphatic alcohols (Wang, Robinson and Lee, "Enhanced Alcohol Production Through On-Line Extraction", Biotechnology and Bioengineering Symp., No. 11, 555-565, 1981); and various polymer systems (International application published under the Patent Cooperation Treaty WO 82/01563, Mattiasson et. al., May 13, 1982). However, it is believed that no truly satisfactory extractants have been discovered, i.e. extractants which enable the process to be operated continuously on a commercial scale at reasonable cost.

SUMMARY OF THE INVENTION

An object of the invention is to provide a process for the production of products by fermentation coupled with liquid-liquid extraction of the products being formed.

Another object of the invention is to provide a new series of extractants which can be used for liquid-liquid extraction of fermentation products from aqueous fermentation media.

Another object of the invention is to provide a process which allows in situ extraction of the product without the need for immobilization of the microorganism.

Yet another object of the invention is to provide a fermentation process which can be operated continuously for a prolonged period of time.

The invention provides a process for producing a product by extractive fermentation in which a substrate is fermented in an aqueous medium by means of a microorganism and the resulting product is removed from the medium by contacting the medium with an extractant for the product which is substantially immiscible in the aqueous medium The invention employs as the extractant a liquid from any one of the following groups:

A. Double bond unsaturated aliphatic alcohols having 12 or more carbon atoms;

B. Saturated branched chain aliphatic alcohols having 14 or more carbon atoms or mixtures thereof (e.g. guerbet alcohols);

C. Double bond unsaturated aliphatic acids having 12 or more carbon atoms;

D. Aliphatic and aromatic mono-, di- or tri-esters having 12 or more carbon atoms, other than dibutyl phthalate;

E. Aliphatic noncyclic ketones and aliphatic aldehydes having 12 or more carbon atoms; and

F. Mixtures of extractants from groups A to E above or mixtures of at least one of the above extractants and at least one other extractant.

The above compounds are substantially non-toxic to most industrially useful microorganisms under the process conditions, tend not to form stable emulsions, have good partition coefficients for common fermentation products, and can be separated from these compounds relatively inexpensively. Hence they combine all the features necessary to make extractive fermentation a feasible commercial process.

Each of the above groups A to E indicates a minimum number of carbon atoms which the extractants may possess. There is no critical maximum number of carbon atoms for each group except that the extractants should of course be liquid under the extraction conditions and the melting points of compounds tend to decrease as the carbon number increases. Accordingly, the practical upper limit of carbon atoms of each group is preferably the maximum number which corresponds to compounds which are liquid at 40° C. Generally, the use of an extractant having a number of carbon atoms close to the minimum for each group is desirable because the product distribution co-efficient tends to decrease with increasing chain length. However, other considerations may be important in the choice of a particular solvent from each group, such as price and availability, so extractants having higher carbon numbers may be preferred in some cases.

The use of the extractant mixtures of Group F above can be particularly advantageous in various circumstances. For example, when more than one product is formed by the fermentation reaction (e.g. during acetone-butanol fermentation), one or more of the components in the extractant mixture may be selective towards a specific product (e.g. acetone). Moreover, extractant mixtures can be employed to adjust and optimize such physical characteristics as the density, boiling range and viscosity of the liquid extractant.

When an extractant other than one of those listed in Groups A to E is used as a component of a mixture according to Group F, the other extractant should be carefully chosen to ensure that it does not impart harmful or disadvantageous characteristics to the extractant mixture, e.g. toxicity to microorganisms or the tendency to form stable emulsions.

Particularly preferred solvents from the above groups are listed below. Some conventional extractants are also listed for comparison and are identified as Group P.

Group A		
(A1)	oleyl alcohol	(cis-9-octadecene-1-ol)
(A2)	phytol	(3,7,11,15-tetramethyl-2-hexadecene-1-ol)

-continued

(A3)	isophytol	(3,7,11,15-tetramethyl-1-hexadecene-3-ol)
Group B		
(B1)	isostearyl alcohol e.g. the commercial product sold under the trademark ADOL 66	
(B2)	isooctyl alcohol e.g. the commercial product sold under the trademark Eutanol G-16	
(B3)	octyl dodecanol e.g. the commercial product sold under the trademark Eutanol G	
Group C		
(C1)	oleic acid	(cis-9-octadecenoic acid)
(C2)	linoleic acid	(9,11-octadecadienoic acid)
(C3)	ricinoleic acid	(12-hydroxy-9-octadecenoic acid)
Group D		
(D1)	dodecyl acetate	($CH_3COO(CH_2)_{11}$)
(D2)	butyl dodecanoate	($CH_3(CH_2)_{10}COOC_4H_9$)
(D3)	dibutyl sebacate	($C_4H_9OOC(CH_2)_8COOC_4H_9$)
(D4)	di(2-ethylhexyl)sebacate	($C_8H_{17}OOC(CH_2)_8COOC_8H_{17}$
(D5)	dibutyl adipate	($C_4H_9OOC(CH_2)_4COOC_4H_9$)
(D6)	di(2-ethylhexyl)adipate	($C_8H_{17}OOC(CH_2)_4COOC_8H_{17}$
(D7)	di(2-ethylhexyl)phthalate	($C_8H_{17}OOCC_6H_4COOC_8H_{17}$)
(D8)	di(3,5,5-trimethylhexyl) phthalate	($C_9H_{19}OOCC_6H_4COOC_9H_{19}$)
(D9)	glycerol tridecanoate	($[CH_3(CH_2)_8COOCH_2]_2CHOCO(CH_2)_8CH_3$)
Group E		
(E1)	2-dodecanone	($CH_3CO(CH_2)_9CH_3$)
(E2)	dodecanal	($CH_3(CH_2)_{10}CHO$)
Group F		
(F1)	the commercial product sold under the trademark ADOL 85 NF ([illegible] percent oleyl alcohol)	
(F2)	the commercial product sold under the trademark ADOL 330 (62 percent oleyl alcohol)	
(F3)	the commercial product sold under the trademark HD oleyl alcohol (commercial oleyl alcohol)	
Group P (prior art extractants)		
(P1)	1-dodecanol	(lauryl alcohol)
(P2)	dibutyl phthalate	($C_6H_4(COOC_4H_9)_2$)
(P3)	tributyl phosphate	($(C_4H_9)_3PO_4$)
(P4)	PPG 1,000	(polypropylene glycol, having an average molecular weight of 1,000)

The Table below lists the pertinent physical data f each of the above compounds, including the conve tional compounds of Group P for comparison. In th Table, the compounds are identified by the letter a number in brackets which precedes each of the co pounds in the above list.

The Table gives the following information for ea compound: (a1) distribution coefficient in fermentati broth, (a2) distribution coefficient in distilled water; (emulsion tendency; (c1) normal boiling point, (c2) mel ing point, (c3) liquid density, (c4) latent heat of vapo zation, (c5) molecular weight, (c6) lethal dose fifty; b ocompatibility data in the form of (d1) percent surviv (d2) percent metabolic activity and (d3) biocompatib ity rating; mutual solubilities (e1) solvent in water, a (e2) water in solvent.

TABLE

PHYSICAL DATA OF THE EXTRACTANTS

	EXTRACTANT								
EXTRACTANT DATA	A1	A2	A3	B1	B2	B3	C1	C2	C3
(a) DEtOH, g/g									
1: in ferm. broth	.289	.306	.157	.192	.152	.127	.171	.209	NA
2: in dist. water	.166	.248	.219	.251	.223	.181	NA	.107	NA
(b) emulsion tendency	1	0	1	0	0	1	1	0	1
(c) 1: NBP, deg. C.	360	355	375	360	300	350	370	395	410
2: MP, deg. C.	+16	<27	<20	+5	−13	<[illegible]	+5	−12	−6
3: DENS, g/ml	.849	.850	.843	.861	.842	.850	.895	.902	.940
4: Hvap, cal/g	49	44	46	45	50	44	72	50	48
5: MOL. WT. cal/g	269	297	297	295	242	299	282	281	298

TABLE-continued

PHYSICAL DATA OF THE EXTRACTANTS

6: LD50, g/kg	26	NA	NA	20	6.4	NA	NA	NA	NA
(d) biocompatibility									
1: % survival	96	100	101	104	103	115	100	90	10
2: % activity	98	105	95	100	97	100	100	102	10
3: rating	B	B	B	B	B	B	B	B	I
(e) mutual solubility									
pct 1: solv. in water	13.5	12.1	12.7	11.9	12.6	10.8	2.23	3.25	32.2
ppb 2: water in solv.	11.3	1.07	1.83	2.73	8.36	0.29	11.6	41.2	385
EXTRACTANT DATA	A1	A2	A3	B1	B2	B3	C1	C2	C3

	EXTRACTANT								
EXTRACTANT DATA	D1	D2	D3	D4	D5	D6	D7	D8	D9
(a) DEtOH, g/g									
1: in ferm. broth	.150	.165	.116	.221	.129	.093	.037	.055	.108
2: in dist. water	.044	.090	.078	.058	.090	.080	.093	.064	.085
(b) emulsion tendency	1	0	1	0	0	1	0	1	1
(c) 1: NBP, dC	280	440	344	470	300	420	384	440	NA
2: MP, dC	<0	<0	<0	−48	−38	−70	−46	−30	+24
3: r, g/ml	.865	.860	1.12	.912	.962	.922	.981	.971	1.00
4: dHvap, cal/g	51	58	41	37	47	39	35	36	NA
5: MOL. WT.	229	256	315	427	258	371	391	419	555
6: LD50, g/kg	NA	NA	16	1.3	13	9.1	6.5	NA	NA
(d) biocompatibility									
1: % survival	62	100	100	100	6	100	100	100	100
2: % activity	105	99	103	96	3	101	101	98	103
3: rating	B	B	B	B	I	B	B	B	B
(e) mutual solubility									
pct 1: solv. in water	5.06	3.73	8.42	7.20	9.51	7.71	4.82	4.87	NA
ppb 2: water in solv.	35.8	10.1	0.97	0.00	86.9	0.01	0.00	0.00	NA
EXTRACTANT DATA	D1	D2	D3	D4	D5	D6	D7	D8	D9

	EXTRACTANT								
EXTRACTANT DATA	E1	E2	F1	F2	F3	P1	P2	P3	P4
(a) DEtOH, g/g									
1: in ferm. broth	.363	.514	.235	.253	.208	.590	.651	.886	.510
2: in dist. water	.114	.277	.278	.265	.268	.477	.112	.096	.401
(b) emulsion tendency	1	1	0	0	0	2	2	1	1
(c) 1: NBP, dC	246	260	330	340	330	256	340	289	NA
2: MP, dC	+21	+12	+10	+22	+10	+23	−40	<0	<0
3: DENS, g/ml	.820	.835	.840	.845	.860	.831	1.05	.972	1.01
4: Hvap, cal/g	77	74	47	49	47	82	64	44	NA
5: MOL. WT.	184	184	267	261	269	186	278	266	1000
6: LD50, g/kg	NA	2.3	26	26	26	13	12	3.0	2.2
(d) biocompatibility									
1: % survival	5	10	100	94	95	25	75	0	85
2: % activity	17	15	103	98	100	3	96	2	78
3: rating	I	I	B	B	B	I	B	T	I
(e) mutual solubility									
pct 1: solv. in water	2.44	1.02	13.5	13.5	13.5	17.7	5.33	NA	NA
ppb 2: water in solv.	2802	713	11.3	11.3	11.3	2214	2.62	NA	NA
EXTRACTANT DATA	E1	E2	F1	F2	F3	P1	P2	P3	P4

NOTES RELATING TO THE TABLE

1. (a) Distribution coefficients were calculated on a mass basis, i.e. as ([EtOH]s/[EtOH]a)/DENS(s), where DENS(s) is the density of the solvent, and a density of unity for the aqueous phase is employed. The Distribution coefficient in the fermentation broth was measured as follows: A shake flask containing 30 ml of a 15% glucose medium was inoculated with yeast cells and allowed to grow for 8 hours. At this time, 10 ml of solvent was added to the growing culture. After 24 hours, when virtually all glucose was converted to ethanol, the ethanol concentration in the aqueous and in the solvent phase was measured. The distribution coefficient in distilled water was measured by equilibrating 5 grams of solvent with 5 ml of a 5% (w/v) ethanol solution in distilled water. It is to be noted that in most cases the distribution coefficient observed in the fermentation broth was higher than that in distilled water. This is attributed to a "salt effect" due to the presence of residual glucose, yeast cells etc. in the fermentation broth. Although these distribution coefficients are for ethanol only, it is safe to say that the corresponding distribution coefficients for acetone and butanol will be an order of magnitude higher.
2. (b) The emulsion tendency was rated according to the following scale: 0 - no emulsion tendency, 1 - some emulsion tendency, and 2 - heavy emulsion tendency.
3. (c) Physical Solvent Data. (1) NBP (Normal boiling

TABLE-continued

PHYSICAL DATA OF THE EXTRACTANTS

point) was taken as direct experimental data or extrapolated from boiling point data at reduced pressure. (2) MP (melting point) was taken as direct experimental data, when available. (3) DENS (density) - direct experimental data. (4) Hvap (latent heat of vaporization) was taken as direct experimental data or was calculated using Trouton's Rule (Hvap = 21°NBP). (5) MOL. WT. - experimental data. (6) LD50 (oral lethal dose fifty for rats) - experimental data.

4. (d) Biocompatibility Indicators. (1) % survival is the percentage of surviving yeast cells (also known as the cell viability) after exposure for 16 hours to solvent under the conditions outlined in note 1. The cell viability was measured either by dilution plating or by using the methylene blue staining technique [Lee et al., Biotechnol. Bioeng. Symp., 11 (3rd. Symp. Biotechnol. Energy Prod. Conserv.), 641–649, 1981]. (2) % activity was measured as the average of the percentage glucose consumed and ethanol formed, relative to a control culture. (3) The biocompatibility of solvents was rated as either B: biocompatible, I: inhibitory, or T: toxic. The specific criteria applied in order to distinguish between these three solvent groups were as follows: If no surviving cells appeared in a plate count after solvent exposure, it was rated as toxic, regardless of what other indicators showed. Among the solvents that allowed cells to survive in a plate count, the ones exhibiting ≦90 percent metabolic activity were rated as inhibitory solvents.
The remaining solvents, exhibiting more than 90 percent metabolic activity after solvent exposure, were rated as biocompatible.
It is to be noted that this toxicity information strictly speaking is for yeast cells only. There is good reason to believe, however, that a solvent that is non-toxic to yeast cells, will be non-toxic towards other microorganisms as well.

5. (e) Mutual Solubilities were calculated from UNIFAC [Magnussen et al., Ind. Eng. Chem. Process Des. Dev., 20(2), 331–339, 1981]. (1) The solubility of solvent in water was expressed as mole percent (pct), while (2) the solubility of water in solvent was expressed in mole parts per billion (ppb).

In particular, the Table above shows that the extractants of the invention have no, or only slight, tendency to form emulsions, acceptable biocompatibility, good distribution co-efficients for the product and low water solubility. Hence, they are ideal extractants for use in the present invention.

Examples of the fermentation processes to which the present invention can be applied include the production of ethanol by the fermentation of the yeast *Saccharomyces cerevisiae* (and the bacterium *Zymomonas mobilis*), the production of acetone/butanol by the fermentation of *Clostridium acetobutylicum*, the production of penicillin by the fermentation of *Penicillium chrysogenum*, the production of citric acid by the fermentation of *Aspergillus niger*, and the production of polysaccharides by the fermentation of *Pullularia pullulans*.

It is a particular advantage of the invention that the solvents listed above can be used for in situ extraction of the product. That is, the solvents may be introduced directly into the fermentor where they remove the product from the fermentation medium as the product is being formed. In situ extraction offers the following advantages, namely (i) decreased fermentor costs due to smaller equipment size (capital cost advantage), (ii) decreased product recovery costs due to larger product concentrations (operating cost advantage), and (iii) decreased medium pre-treatment and waste treatment cost due to smaller aqueous flows (operating cost ad vantage).

Since the extractants have little tendency to for stable emulsions with the fermentation medium in th presence of microorganisms, there is no need to imm bilize the microorganism or to separate the microorga ism from the fermentation medium before the extractio takes place.

Moreover, since the extractants separate fairly easil from the aqueous fermentation medium, they can easil be removed from the fermentor and replenished, so th the extraction process may be carried out continuousl In a particularly preferred form of the invention, th extractant/product solution removed from the fermer tor can be treated to separate the product from th extractant and the extractant can then be recycled to th fermentor for the extraction of further product.

The easy separation of the fermentation medium an extractant also permits the fermentation medium to b withdrawn from the fermentor and fresh substrate sol tion to be added, if this is desired.

The fermentation process may accordingly be mad entirely continuous in that the extractant may be rec cled between the fermentor and the separation appar tus and the fermentation medium may be continuousl refreshed. Since the end product is not allowed to rea the critical toxic concentration, the fermentation proc dure can be carried out indefinitely, or at least for a considerable period of time.

The process is advantageously carried out in a continuous stirred tank fermentor in which the microorganism cells are freely suspended. This allows a high yield to be obtained while utilizing well-proven fermentation technology. In such an apparatus, for steady state operation, the input rate of fresh substrate solution (known as the dilution rate, i.e. the input flow rate divided by the volume of the fermentor) should be such that the rate of cell removal in the outflowing medium is equal to the rate of cell production in the fermentor. In this way the microorganism cell population remains virtually unchanged.

The fact that the present invention permits the use of freely suspended cells in the fermentor is advantageous because free cells may be more productive then immobilized cells since free cells do no encounter the mass transfer resistance often associated with immobilization matrices, and the use of immobilized cells tends to make the process expensive. Moreover, since the extractants are non-toxic and do not form stable emulsions in the presence of the microorganisms, steps do not have to be provided to ensure that cells are removed from the fermentation medium before the liquid-liquid extraction takes place

The normal conditions under which the fermentation/extraction procedure takes place are as follows (a) 20°–80° C., (b) vacuum to slightly above atmospheric pressure (0.05–5 atm), (c) pH 3.0–8.0, (d) agitation 0–1000 rpm, (e) aeration 0–5 vvm (liters air/liters fermentation broth-minute), (f) feed concentration 20–800 g/L glucos equivalents, (g) solvent dilution rate 0.1–25 h^{-1}, they should not be considered limitative of the present invention.

While continuous fermentation employing a continuous stirred tank fermentor as indicated above is much preferred, the solvents disclosed herein may also be used with batchwise fermentation, or fed-batch fermentation or immobilized cell fermentation if this is desired.

Furthermore, downstream (rather than in situ) extraction may be carried out, if desired. In such a process, the liquid-liquid extraction of the product is carried out on fermentation medium removed from the fermentor. After separation of the aqueous medium from the extractant/product solution, the medium may be recycled to the fermentor, discarded or treated for the removal of any remaining product.

During the extraction step, the extractant is preferably brought into intimate contact with the aqueous medium in order to promote rapid and complete partition of the product. For example, in the case of in situ extraction, the extractant may be introduced in small streams at the bottom of the fermentor and allowed to rise to the surface to form a continuous surface layer.

After separation of the extractant/product solution from the fermentation medium, the product can be removed from the extractant by any suitable means and, as mentioned above, the extractant may then be recovered and reused for further product extraction. For example, the extractant/ product solution may be distilled in order to separate the product from the extractant. Distillation of the extractant solution does not usually require as much energy input as distillation of the fermentation medium itself because the organic extractants have smaller latent heats of vaporization and heat capacities than water, and because the boiling points of the solvents are much higher than that for water, resulting in fewer equilibrium distillation stages. Furthermore, an extractant can easily be chosen which does not form an azeotrope with the product or otherwise affect the product, so that a product of greater purity can be obtained than is often the case with direct distillation of the fermentation medium.

As an alternative to distillation, the product may be separated from the extractant by stripping with air or CO_2, followed by product condensation, or by any other suitable method.

Preferred apparatus for carrying out the invention is described below with reference to the accompanying drawings, in which:

FIG. 1 is a cross-sectional view of a continuous stirred tank fermentor being operated according to a preferred form of the invention;

FIG. 2 is a representation of a distillation apparatus for separating the extractant and product;

FIG. 3 is a representation of an extractant stripping apparatus which can be used as an alternative to the distillation apparatus of FIG. 3;

FIG. 4 is a schematic diagram representing an integrated apparatus for continus fermentation, product removal and product isolation;

FIG. 5 is a schematic diagram illustrating a preferred, alternative embodiment of the apparatus illustrated in FIG. 4;

FIG. 6 is a cross sectional view of one embodiment of a broth separator used in the apparatus in FIG. 5; and

FIG. 7 is a sketch of an alternative broth separator used in the apparatus of FIG. 5.

The preferred embodiment described below involves the preparation of ethanol by fermentation of a substrate (such as glucose) in the presence of yeast.

FIG. 1 shows an example of a continuous stirred tank fermentor 10 suitable for use in the present invention. The fermentor comprises a container 11 having an overflow outlet 12 and lid 13. During use, a layer 14 of extractant overlies an aqueous fermentation medium 15 containing a yeast capable of converting a substrate such as glucose into ethanol. The container 11 may be provided with a mechanical stirrer (not shown), although such a stirrer is not really necessary as will be apparent from the description below.

Fresh medium containing the substrate is introduced into the fermentor on a continuous basis via a pipe 16. The medium, and possibly entrained extractant, is withdrawn from the fermentor on a continuous basis via pipe 17. Since the rates of introduction and removal of the medium ar the same, the surface level of the medium in the fermentor remains essentially unchanged.

Extractant and air (the input of air may not be required for anaerobic fermentations such as those producing ethanol, acetone and butanol) are introduced into the fermentor via a pipe 18 from separate feed pipes 19 and 20, respectively. A dispersion head 21 is located at the bottom of the fermentor and is connected to the extractant/air pipe 18. The dispersion head is provided with a larger number of small holes so that the extractant and air are separated into small streams as they enter the fermentation medium. Since the extractant and air are less dense than the aqueous fermentation medium, both rise to the surface and, as they do so, come into intimate contact with the fermentation medium. The rising air bubbles have the effect of stirring the medium. The extractant removes a portion of the ethanol product from the aqueous fermentation medium as the product is being formed so that the concentration in the fermentation medium itself rarely exceeds 2 to 3% (w/v). In this way, the concentration of ethanol in the aqueous fermentation medium never reaches the 11-12% (w/v) level at which end product inhibition causes the reaction to cease. Carbon dioxide generated during the fermentation also helps to stir the fermentation medium as the gas bubbles rise to the surface.

Since the extractant has little tendency to form an emulsion with the fermentation medium, the layer 14 is substantially free of aqueous medium and can be decanted through the outlet 12. Air and carbon dioxide also leave the fermentor through this outlet. The extractant, which contains the extracted ethanol, then undergoes treatment so that the extractant and ethanol may be removed, as will be explained below.

Yeast cells are removed from the fermentor with the aqueous medium, but the rate of removal may be made such that it is essentially the same as the increase in the yeast cell population as the fermentation proceeds. If desired, however, the yeast cells in the medium removed from the fermentor may be recycled to the fermentor.

Since the fermentor 10 is continuously supplied with substrate and oxygen and since the product is continuously removed to avoid end product inhibition or a reduction of the production rate, the fermentation may proceed indefinitely.

FIGS. 2 and 3 are representations of apparatus that may be used to separate the ethanol from the extractant. FIG. 2 shows a distillation apparatus 25 having an extractant solution inlet 26, an upper outlet 27 for the ethanol and a lower outlet 28 for the extractant. The apparatus itself is conventional, so further details need not be provided.

FIG. 3 shows an apparatus 30 for stripping the ethanol product from the extractant. The extractant/ethanol solution enters at inlet 31 and flows downwardly through the apparatus to an outlet 32. Air or carbon dioxide is introduced into the apparatus through an inlet 33 at the bottom of the apparatus and rises in intimate contact with the descending solution to an outlet 34 at the top of the apparatus. The air or CO_2 removes the ethanol in the form of vapour from the extractant solution.

FIG. 4 shows an integrated apparatus for the continuous production of ethanol by fermentation and liquid-liquid extraction. Where appropriate, the parts of the apparatus illustrated in the previous figures are identified by the same reference numerals.

Fresh medium containing the substrate is introduced into fermentor 10 via aqueous feed line 40. Extractant is introduced into the bottom of the fermentor via extractant feed line 41 and the extractant/ethanol/water mixture is withdrawn via feed line 42. The medium is continuously withdrawn from the fermentor 10 via outlet 43. The extractant/ethanol/water mixture from line 42 is fed to an extractant separator 30. The separated extractant is recycled to extractant feed line 41 via line 44, and the separated ethanol/water mixture is fed to a distillation column via line 45.

The medium extracted from the fermentor 10, which is mainly a mixture of ethanol and water, is fed to a beer stripper 46 which is similar to extractant separator 30. The mixture is separated into a 50% ethanol water solution and a waste water component. The waste water component is discharged through pipe 47 and the 50% ethanol solution is fed to the distillation column 25 via pipe 48.

The ethanol distillation column separates the incom ing feeds into azeotropic ethanol, which exits throug pipe 49, and water, which exits via pipe 50.

During the complete conversion of concentrate substrate feeds (>300 g/L) some association betwee the extractant and the fermentation broth is unavoidabl because the broth becomes entrained into the extractar due to the vigorous evolution of carbon dioxide. Thi phenomenon may lead to a significant loss of fermentation broth in the extractant stream 42 leaving the fermentor, because the initial water concentration in th aqueous feed 40 is relatively low when concentrate substrate feeds are employed. Thus, it is desirable t control the amount of water in the effluent extractan stream 42. FIG. 5 shows a preferred embodiment of th invention for the production of ethanol by extractiv fermentation using concentrated substrate feeds, i which the effluent extractant stream 42 enters a brot separator 51, and is separated into a broth stream 5 (consisting largely of microbial cells in water), which i returned to the fermentor 10, and an extractant/ethanol/water stream 52, which is further processed i the extractant separator 30.

FIG. 6 shows one embodiment of the broth separato 51 in the form of a gravity settler comprising a pluralit of individual settling chambers arranged in series on below the other, into which the extractant/ethanol/broth stream 42 is separated into stream 53 extracte from the bottom of each chamber, consisting largely o fermentation broth, and stream 52 extracted as a overflow from the top of each chamber, consisting largely o extractant and ethanol. The separation efficiency of thi device is defined by the size and number (n) of th individual settling chambers, and by the flowrate o stream 53, which is returned to the fermentor.

FIG. 7 shows an alternative embodiment of the broth separator 51 in the form of a centrifuge, in which the extractant/ethanol/broth stream 42 is separated int stream 53, consisting largely of fermentation broth, an stream 52, consisting largely of extractant and ethanol The separation efficiency of this device is defined by th equipment size, and by the rotation of the centrifuge.

The apparatus thus operates continuously for th production of ethanol while avoiding the problem o end product inhibition.

EXAMPLES

As noted above, a particular advantage of the presen invention is that it permits the liquid-liquid extraction to be carried out in situ in a continuous stirred tank fermentor.

To determine the technical and economic advantage of such a process various experiments were carried ou as described below. The abbreviations used in the description of these experiments are given in the followin nomenclature:

Nomenclature	
Conv.	Glucose conversion = $(S_o - S)/S_o*100$
DENS	Density, g/ml
DEtOH	Ethanol distribution coefficient, $[EtOH]_s/[EtOH]_w$
Ds	Extractant dilution rate = Fs/V, h^{-1}
Dw	Aqueous dilution rate = Fw/V, h^{-1}
EE	Extraction efficiency = PDs/PDtot*100
Fs	Extractant flowrate, L/h
Fw	Medium flowrate, L/h
Hvap	Latent heat of vaporization at normal boiling point, cal/g
LD50	Lethal dose fifty = the dosage of solvent that will

-continued

Nomenclature	
	kill 50% of a rat population, in g solvent/kg rat
MP	Melting point, deg. C.
NBP	Normal boiling point, deg. C.
Pw	Effluent aqueous ethanol concentration, g/L
Ps	Effluent ethanol concentration in solvent, g/L
PDa	Aqueous ethanol productivity = Pw*Dw, g/L-h
PDs	Extractant ethanol productivity = Ps*Ds, g/L-h
PDtot	Total ethanol productivity = PDa + PDs, g/L-h
So	Initial glucose concentration, g/L
S	Effluent aqueous glucose concentration, g/L
V	Working volume of fermentor = volume of aqueous phase, L
X	Effluent aqueous cell concentration, g/l

The equipment employed as shown in FIG. 4 of the drawings.

Demonstration of Technical Advantages

Below are shown data for two experiments with in situ extractive fermentation of a 159 g/L glucose feed to ethanol in a continuous stirred tank fermentor with a working volume of 1 liter. The microorganism used was the yeast Saccharomyces cerevisiae NCNY 716. The experimental data have been compared to data obtained using an advanced computer model developed by the authors [Kollerup and Daugulis, Biotechnology & Bioengineering, September 1985].

The technical feasibility of extractive fermentation in a CSTF (Continuous Stirred Tank Fermentor) as well as the adequacy of the model are clearly evident from these data. It is particularly important to note the improvement in system performance when operating in the extractive fermentation mode, as measured by % Conversion, and total ethanol productivity (PDtot).

Experimental and Predicted Performance Data for Conventional and Extractive Fermentations in a Continuous Stirred Tank Fermentor.

PARAMETER	EXPERIMENT #1	EXPERIMENT #2
So, g/L	159	159
Dw, h-1	0.168	0.220
Ds, h-1	1.067	3.217
DEtOH, g/g	0.174	0.110
pH	4.0	4.0
temperature, deg. C.	30.0	30.0
agitation speed, RPM	100	100

	OPERATION							
	Conventional		Extractive		Conventional		Extractive	
	PRED	EXP	PRED	EXP	PRED	EXP	PRED	EXP
S, g/L	76.8	84.1	15.6	10.3	106.7	102.3	43.5	27.8
X, g/L	5.1	4.7	9.4	9.9	3.4	2.9	7.9	10.1
Pw, g/L	36.6	33.4	31.5	32.3	23.6	23.2	21.0	27.8
Conv., %	51.7	47.1	90.2	93.5	32.9	35.7	72.6	82.5
PDtot, g/L-h	5.8	5.6	11.2	11.3	5.2	5.5	12.1	13.3
Ps, g/L	—	—	5.5	5.5	—	—	2.3	2.6
EE, %	—	—	52.7	52.0	—	—	61.8	61.5

Demonstration of Economic Advantages

In the following, three different operating conditions of a CSTF were considered, namely (1) Conventional CSTF with 15% glucose feed, (2) Extractive CSTF with 15% glucose feed, and (3) Extractive CSTF with 50% glucose feed. These three conditions were simulated with the above mentioned computer model, since the model evidently agreed well with experimental data. The aqueous dilution rate under the three conditions was the dilution rate giving the maximum total ethanol productivity.

Simulated Fermentation Data For Three Different Operating Conditions.

. PARAMETER	OPERATING CONDITION (1)	(2)	(3)
So, g/L	150	150	500
Dw, h-1	0.077	0.167	0.149
S, g/L	2.6	10.0	10.0
X, g/L	10.8	10.8	45.9
Pw, g/L	67.3	30.3	36.7
Conversion, %	98.3	93.3	98.0
PDtot, g/L-h	5.2	11.2	42.5
Ds, h-1	—	1.0	5.0
Ps, g/L	—	6.1	7.3
DEtOH, g/g	—	0.2	0.2
EE, %	—	54.6	87.1

Approximate mass balances were calculated for the above three operating conditions and costs were projected based on (i) medium pretreatment and waste treatment costs, (ii) ethanol recovery costs, and (iii) fermentor cost. In this analysis, the following assumptions were made:

Medium pretreatment and waste treatment costs are directly proportional to the amount of water in the medium and waste stream, respectively.

Ethanol recovery costs are calculated for the distillation to azeotropic ethanol (95% w/w) from the combination of the streams from lines 43 and 45. [The energy of distillation is taken from R. C. Righelato, "Anaerobic Fermentation: Alcohol Production", Trans Roy. Soc. London B, 290-303 (1980)].

The fermentor cost is assumed to be proportional to $(V)^{0.6}$, where V is the working volume of the fermentor.

RELATIVE COSTS ASSOCIATED WITH DIFFERENT OPERATING CONDITIONS.

	(1)	(2)	(3)
water in the aqueous feed	72.1	75.1	17.5
waste water from beer stripper	66.9	70.9	14.2
TOTAL WATER (Waste + Pretreatment)	139.0	145.8	31.7
W/P COST relative to (1)	1.00	1.05	0.70
STREAMS to Ethanol Distillation Column			
water	72.1	75.1	17.5

-continued

RELATIVE COSTS ASSOCIATED WITH DIFFERENT OPERATING CONDITIONS.

	(1)	(2)	(3)
ethanol	5.2	5.2	5.2
TOTAL (distil. column feed)	77.3	80.[illegible]	22.7
percent ethanol in STREAM	6.7%	6.5%	22.9%
energy of distillation/ gross ethanol combustion energy	0.25	0.26	0.175
DISTILLATION COST relative to (1)	1.00	1.04	0.70
Fermentor volume (L)	1.00	0.464	0.122
FERMENTOR COST relative to (1)	1.00	0.63	0.28

From the above analysis it can be seen that extractive fermentation provides substantial cost savings, particularly when concentrated glucose feeds are fermented. The use of the extractants of the invention makes extractive fermentation in a CSTF feasible.

Although preferred embodiments of the invention have been described above various modifications and alternatives to the above embodiments will be apparent to persons skilled in the art. All such modifications and alternatives form part of this invention provided they do not depart from the scope thereof as defined by the following claims.

We claim:

1. A process for the continuous production of a product selected from ethanol, penicillin, citric acid and polysaccharides by fermentation with a microorganism capable of producing said product and being selected from *Saccharomyces cerevisiae*, ~~or~~ *Zymomonas mobilis*, *Penicillium chrysogenum*, *Aspergillus niger* and *Pullularia pullulans* respectively, which process comprising culturing said microorganism in an aqueous fermentation medium in a fermentation vessel while continuously introducing an aqueous solution containing a substrate for the microorganism into said fermentation vessel and continuously removing said product from said medium by contacting said medium in situ with a continuiously introduced liquid which is an extractant for said product but which is substantially immiscible with said aqueous medium, and wherein said extractant is fully biocompatible with the selected microorganism and is at least one compound selected from the group consisting of (A) double bond unsaturated aliphatic alcohols having 12 or more carbon atoms; (B) saturated branched chain aliphatic alcohols having 14 or more carbon atoms or mixtures thereof; (C) double bond unsaturated aliphatic acids having 12 or more carbon atoms; (D) aliphatic and aromatic mono-, di- or tri-esters having 12 or more carbon atoms, other than dibutyl phtalate; (E) aliphatic noncyclic ketones and aliphatic aldehydes having 12 or more carbon atoms; and (F) mixtures of extractants from groups (A) to (E) above or mixtures of at least one of the above extractants and at least one other extractant.

2. A process according to claim 1 wherein said extractant is selected from the group consisting of oleyl alcohol, phytol, isophytol, isostearyl alcohol, isocetyl alcohol, octyl dodecanol, oleic acid, linoleic acid, ricinoleic acid, dodecyl acetate, butyl dodecanoate, dibutyl sebacate, di(2-ethylhexyl)sebacate, dibutyl adipate, di(2-ethylhexyl)adipate, di(2-ethylhexyl)phthalate, di(3,5,5-trimethylhexyl)phthalate, glycerol tridecanoate, 2-dodecanone and dodecanal.

3. A process according to claim 1 wherein said fermentation vessel is a continuous stirred tank fermentor.

4. A process according to claim 1 wherein said microorganism is freely suspended in said fermentation medium.

5. A process according to claim 1 wherein, after contacting the fermentation medium with said extractant, said extractant is separated from the medium and subjected to a procedure for separation of the product from the extractant.

6. A process according to claim 5 wherein said procedure comprises distillation of said extractant.

7. A process according to claim 5 wherein said procedure comprises stripping of the product from the extractant by contact with a gas, followed by condensation of the product.

8. A process according to claim 5, wherein, after said procedure, the extractant is recirculated into said fermentation vessel containing said fermentation medium.

9. A process according to claim 5 wherein medium entrained in said extractant separated from the medium is stripped from said extractant prior to said procedure.

10. A process according to claim 9 wherein said medium stripped from said extractant is recirculated to a fermentation vessel containing said fermentation medium.

11. A process according to claim 1 wherein the microorganism is either *Saccharomyces cerevisiae* or *Zymomonas mobilis*, and the product is ethanol.

12. A process for preparing a product selected from ethanol, penicillin, citric acid and polysaccharides by fermentation with a microorganism capable of producing said product and being selected from *Saccharomyces cerevisiae* or *Zymomonas mobilis*, *Penicillium chrysogenum*, *Aspergillus niger* and *Pullularia pullulans* respectively, which process comprises:

continuously introducing an aqueous solution containing a substrate for the microorganism into a continuous stirred tank fermentor containing an aqueous fermentation medium and said microorganism in freely suspended form;

continuously withdrawing said aqueous fermentation medium from the fermentor at a rate substantially equal to the rate of introduction of said substrate solution;

continuously introducing a liquid into said fermentation to contact the fermentation medium, said liquid being an extractant for the product selected from the group consisting of (A) double bond unsaturated aliphatic alcohols having 12 or more carbon atoms; (B) saturated branched chain aliphatic alcohols having 14 or more carbon atoms or mixtures thereof; (C) double bond unsaturated aliphatic acids having 12 or more carbon atoms; (D) aliphatic and aromatic mono-, di- or tri-esters having 12 or more carbon atoms, other than dibutyl phthalate; (E) aliphatic noncyclic ketones and aliphatic aldehydes having 12 or more carbon atoms: and (F) mixtures of extractants from groups (A) to (E) above or mixtures of at least one of the above extractants and at least one other extractant;

continuously withdrawing said liquid from the fermentor after said contact with the fermentation medium at a rate substantially equal to the rate of introduction of the liquid into the fermentator; and

separating said product from the liquid withdrawn from the fermentor and recirculating the remaining liquid to the fermentor as at least part of the liquid being introduced therein.

* * * * *